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~~2-Thio~~ (2-HYDROXYETHOXY)METHYL NUCLEOSIDE ANALOGUES
A. Models in the Study of the Acid Catalysed Hydrolysis of the Glycosyl Bond
B. Evaluation of Biological Activities

University — Université
UNIVERSITY OF ALBERTA

Degree for which thesis was presented — Grade pour lequel cette thèse fut présentée
Ph.D

Year this degree conferred — Année d'obtention de ce grade
1984

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(2-HYDROXYETHOXY)METHYL NUCLEOSIDE ANALOGUES.

A. MODELS IN THE STUDY OF THE ACID CATALYSED HYDROLYSIS OF THE GLYCOSYL BOND.

B. EVALUATION OF BIOLOGICAL ACTIVITIES.

BY

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF CHEMISTRY

EDMONTON, ALBERTA

SPRING, 1984

THE UNIVERSITY OF ALBERTA

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TITLE OF THESIS: (2-Hydroxyethoxy)methyl Nucleoside Analogues.

A. Models in the Study of the Acid Catalysed Hydrolysis of the Glycosyl Bond.

B. Evaluation of Biological Activities.

DEGREE FOR WHICH THESIS WAS PRESENTED: Ph.D.

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A. MODELS IN THE STUDY OF THE ACID CATALYSED HYDROLYSIS OF THE GLYCOSYL BOND.

B. EVALUATION OF BIOLOGICAL ACTIVITIES.

submitted by PETER WILLIAM HATFIELD in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Chemistry.

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External Examiner

Date 18th November 1983

To my family

ABSTRACT

Treatment of 1,3-dioxolane with acetyl bromide gave a good yield of (2-acetoxyethoxy)methyl bromide. Silylated 5-substituted uracils and cytosines were coupled with this alkylating agent in acetonitrile to give good yields of the desired N-1 isomers. For example alkylation of 5-fluorouracil gave an 84% yield of 1-[(2-acetoxyethoxy)-methyl]-5-fluorouracil.

A similar high yielding procedure was found for the coupling of chloropurines in benzene with the presence of mercury (II) cyanide being required for exclusive N-9 alkylation. Further modifications of these initial products allowed the synthesis of some biologically interesting molecules, including 9-[(2-hydroxyethoxy)-methyl]adenine, 2-amino-6-chloro-9-[(2-hydroxyethoxy)-methyl]purine and 9-[(2-hydroxyethoxy)methyl]guanine. Halogenation of the 8-position of 9-[(2-hydroxyethoxy)-methyl]guanine gave good yields of 8-bromo-9-[(2-hydroxy-ethoxy)methyl]guanine, 8-chloro-9-[(2-hydroxyethoxy)-methyl]guanine and 9-[(2-hydroxyethoxy)methyl]-8-iodo-guanine. The 8-bromo compound was used in the preparation of various 8-substituted derivatives of 9-[(2-hydroxy-ethoxy)methyl]guanine including 8-hydroxy-9-[(2-hydroxy-ethoxy)methyl]guanine, 8-amino-9-[(2-hydroxyethoxy)-

methyl]guanine and some 8-alkylamino-9-[(2-hydroxyethoxy)-methyl]guanines. Reaction of 9-[(2-hydroxyethoxy)methyl]-guanine with methyl radicals and t-butyl radicals gave samples of 9-[(2-hydroxyethoxy)methyl]-8-methylguanine and 8-t-butyl-9-[(2-hydroxyethoxy)methyl]guanine respectively.

The activities of these nucleoside analogues against herpes simplex virus (I and II), vesicular stomatitis virus and vaccinia virus were assessed in primary rabbit kidney cells. Almost all of the 9-[(2-hydroxyethoxy)-methyl]purines showed significant inhibition of viral proliferation. Substituents at the 8-position of the 9-[(2-hydroxyethoxy)methyl]guanine caused a decrease in antiviral activity which appeared to be related to the size of the substituent. The 8-amino, bromo, iodo and methyl compounds were found to be more selective in their action as judged by the ratio of the IC_{50} for incorporation of thymidine (or 2'-deoxyuridine) into DNA of uninfected cells, to that for inhibition of herpes simplex virus (I or II).

The 1-[(2-hydroxyethoxy)methyl]pyrimidines were devoid of significant antiviral activity. They did not potentiate the antitumor activity of 5-fluoro-2'-deoxyuridine and its cogeners.

The acid catalysed hydrolyses of these (2-hydroxyethoxy)methyl nucleosides were studied in 1 M HCl at

77°C. In all cases the hydrolyses occurred at slower rates than those of the naturally occurring nucleosides due to the greater instability of the proposed oxocarbonium ion (or Schiff base) intermediate. Activation parameters, pH/rate profiles, specific acid catalysis and the effects of substituents in the purine series were all consistent with the widely accepted oxocarbonium ion mechanism.

Although the slow rate of hydrolysis of the 1-[(2-hydroxyethoxy)methyl]cytosines allowed deamination to compete, the rate of hydrolysis appeared to be related to the basicity of the leaving group. This was confirmed by the relative rates of hydrolysis of 3-[(2-hydroxyethoxy)-methyl]cytosine, 1-[(2-hydroxyethoxy)methyl]isocytosine and 1-[(2-hydroxyethoxy)methyl]pyrimidin-4-one.

In contrast electron-withdrawing groups at the 5-position of 1-[(2-hydroxyethoxy)methyl]uracil caused decreases in the hydrolysis rate. Comparison of the relative rates strongly suggest a changeover in mechanism in this series. This was further supported by the trapping of a Schiff base intermediate in anhydrous methanolic hydrogen chloride. These results provide insight into the anomalous behaviour of pyrimidine ribonucleosides. A general scheme for the hydrolysis of all nucleosides is proposed.

ACKNOWLEDGEMENTS

I would like to thank the faculty members and my colleagues in the chemistry department who made my stay a rewarding experience.

I am grateful to Dr. M.J. Robins for his patience and encouragement whilst directing this work.

Special thanks are due to the support staff for their help and good humour, especially Ms. Diane Dowhaniuk who typed this manuscript.

Financial support from the Alberta Heritage Foundation for Medical Research and the University of Alberta is gratefully acknowledged.

My warmest thanks go to the many good friends I have known in Edmonton who supported me during difficult times.

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INTRODUCTION

It is well known that genetic information is contained in deoxyribonucleic acid (DNA) and that the translation of this genetic code into functioning proteins occurs via ribonucleic acids (RNA).[1] Thus nucleic acids occupy a central position in cellular metabolism which is summarised in Figure 1.

Nucleic acids are 3',5' phosphodiester linked polymers of adenine (1), guanine (2), cytosine (3), thymine (4) and uracil (5) nucleosides and have been found to have molecular weights up to several billion. Complete copies of all the DNA have to be produced by the cell before mitotic cell division can occur and material is being synthesised constantly by a number of pathways. Blockage of these biosynthetic pathways is therefore one important target for the inhibition of rapidly dividing cells, such as in tumors. It is possible that a synthetic antimetabolite, though toxic to normal cells, can show a favourable therapeutic index in the treatment of diseases involving rapid cell division. 5-Fluorouracil (14) is an example of this.[2] After being metabolised to the active 5-fluoro-2'-deoxyuridylic acid (15) it strongly inhibits thymidylate synthetase, the enzyme responsible for the only de novo

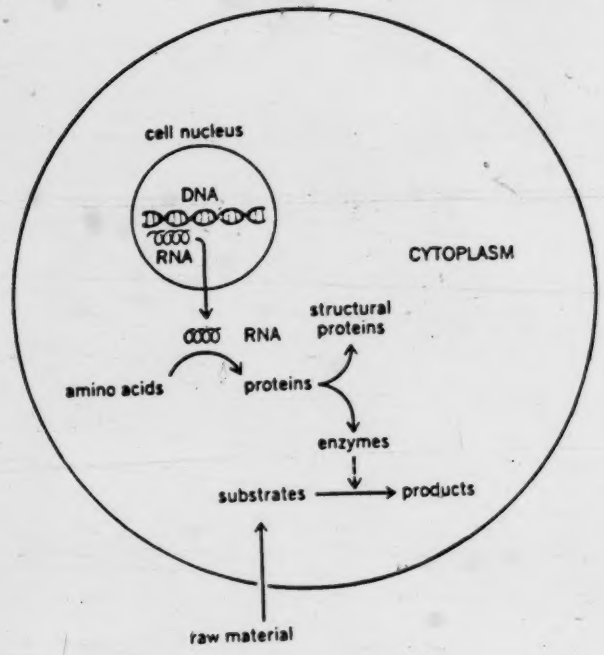
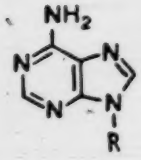


Figure 1: summary of cell metabolism



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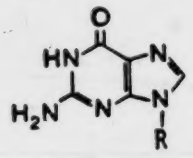

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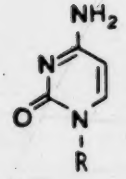

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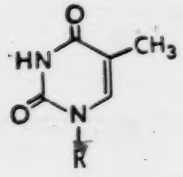

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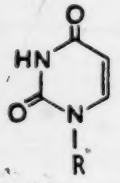

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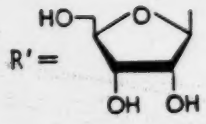

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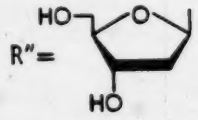
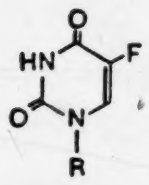


2'-DEOXY-β-D-RIBOFURANOSYL

(2'-DEOXY-β-D-ERYTHRO-PENTOFURANOSYL)

production of thymidylic acid (16), an essential component of DNA. This type of antimetabolite is



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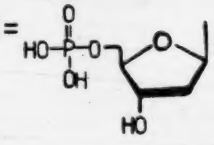
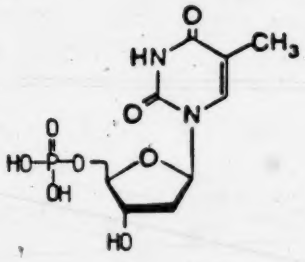
(15) R= 



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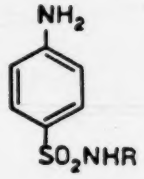
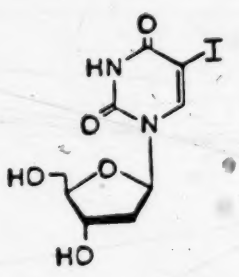


(17)

hardly the "Magic Bullet" Ehrlich spoke of since normal cells are affected also.[3] The antibacterial sulphonamides (17) are better examples of this concept, since they inhibit bacterial production of folic acid but do not affect human cells whose only source of folic acid is dietary.[4]

Antiviral chemotherapy offers similar possibilities. Although viruses utilise the "machinery" of the cell they infect, they have genes that code for enzymes of their own. These viral enzymes may differ from their cellular counterparts in such properties as heat stability, pH and salt requirements and substrate specificity. Therefore they are potential targets for

antimetabolites. Several viral coded enzymes have been identified such as: thymidine and deoxycytidine kinases,[5] DNA polymerases,[6] nucleoside phosphotransferases,[7] deoxycytidine deaminase[8] and deoxycytidine monophosphate deaminase.[9] Many nucleoside analogs have been synthesised and a variety of antiviral activities have been observed.[10] Some, including 5-iodo-2'-deoxyuridine (18), 5-trifluoromethyl-2'-deoxyuridine (19) and adenine arabinoside (20), have been used in the treatment of viral infections in humans.



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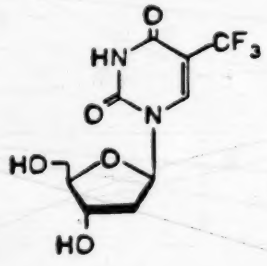

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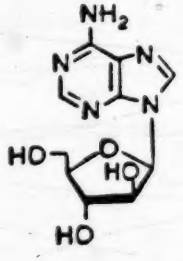

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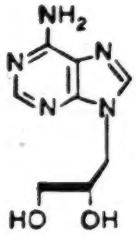
However, serious side effects have been reported with the systemic use of these drugs, for example (18) and (19) can be immunosuppressive, mutagenic, embryotoxic and possibly carcinogenic.[11,12] Clearly there is a need for superior antimetabolites.

Recently there has been widespread interest in the replacement of the sugar moiety with acyclic groups that resemble portions of the cyclic sugar.[13-24] Of these the two with the most pronounced antiviral activity are 9-(2,3-dihydroxypropyl)adenine (21) and 9-[(2-hydroxyethoxy)methyl]guanine (22). The former was found to inhibit the replication of a number of DNA and RNA viruses,[24,25] with host cytoxicity appearing at



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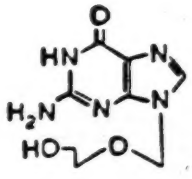

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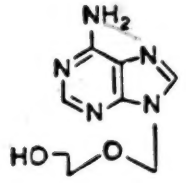
doses far in excess of those required for viral inhibition.[24] Acycloguanosine (22), more commonly known by the Burroughs-Wellcome name of "acyclovir", has generated much excitement resulting in a large number of investigations into its' potent antiherpes virus activity. This new antimetabolite is the subject of the following chapter.

"Acyclovir" 9-[(2-hydroxyethoxy)methyl]guanine (22)

The first (2-hydroxyethoxy)methyl nucleoside was reported by Schaeffer[26] who was investigating the structural binding requirements of the enzyme adenosine deaminase.[27-30] It had been established that the 2' and 3' hydroxyl functions were not of critical importance,[31-38] but the 5' hydroxyl group had to be present for substrate activity.[39-41] It was expected, and found,[26] that 9-[(2-hydroxyethoxy)methyl]adenine (23) was a substrate for adenosine deaminase. This



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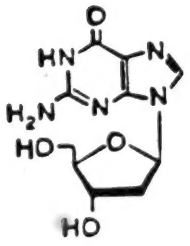
result suggested that this type of nucleoside analogue might be recognised by other enzymes. In 1977 the synthesis and potent antiviral activity of (22) was reported.[42,43] The authors reported that (22) had an effective dose for 50% inhibition of viral activity

(ED_{50}) which was ten-fold smaller than that of the clinically used (18) and (20), and that (22) was essentially non-toxic to Vero cells. Collins and Bauer noted that the sensitivities of herpes simplex virus type I and II to acyclovir were the same.[44] Equivalent sensitivities also were found by De Clercq,[45] but not by Crumpacker who reported HSV-II to be ten times less sensitive.[46] This may be due to the differential sensitivities in different cell lines.[44,48] Varicella-Zoster virus (VZV),[43,45,47] Epstein-Barr virus (EBV)[49,50a] and herpes B virus[51] also were reported to be inhibited by acyclovir. Vaccinia and a range of RNA viruses were not inhibited.[43] Cytomegalovirus (CMV) was inhibited only by high concentrations of the drug.[46,62]

Elion showed that in HSV-I infected Vero cells radiolabelled acyclovir was converted to mono, di and triphosphates.[42,53,54] In uninfected or vaccinia virus infected cells, only a very small degree of phosphorylation was observed. Surprisingly, the enzyme responsible for the initial phosphorylation was found to be the viral coded thymidine kinase and not a guanosine kinase.[42,44,47,53-55] Why acyclovir behaves as a substrate for this pyrimidine nucleoside enzyme is still unclear, especially since it is more efficiently

phosphorylated than either 2'-deoxyguanosine (11) or 1-[(2-hydroxyethoxy)methyl]thymine (24).[54-56]



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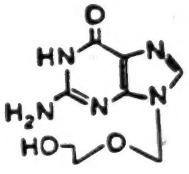

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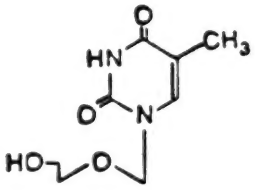


(24)

The second and third phosphorylations of acyclovir monophosphate were shown to be effected by host cell guanylate kinases.[53,55] Acyclovir triphosphate was found to be the active antimetabolite. This triphosphate inhibits the viral DNA polymerase with an inhibition constant thirty times less than that for the host cell polymerase.[53,55,57-59] Acyclovir triphosphate can act as a chain terminator of growing DNA if it is utilised as a substrate. This has been shown to occur[60-64] and the acyclovir terminated template binds very tightly to the polymerase.[63] The authors also showed that the repair 3'-5' exonuclease would not excise the acyclovir moiety.

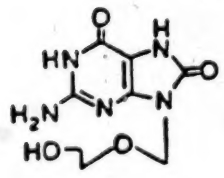
The high selectivity of acyclovir is due to two factors: firstly, phosphorylation of the drug occurs to an appreciable extent only in virus-infected cells (once phosphorylated efflux across the cell membrane is difficult[53]). Secondly, the viral DNA polymerase is thirty times more sensitive to acyclovir triphosphate than is the host cell DNA polymerase.

The combination of these two factors results in a 300-3,000 fold difference in toxicity toward normal versus infected cells. The importance of the thymidine kinase activity was further shown by work on cell lines which were transformed biochemically to contain the HSV-I thymidine kinase gene. These cells were found to be much more sensitive to acyclovir than were the wild type.[61,65,66] Field took this investigation one step further and studied the effect of acyclovir on normally resistant viruses (such as vaccinia, pseudorabies and TK^- HSV-I mutants) in these TK^+ transformed cells. In all cases a dramatic increase in inhibition sensitivity was observed.[67,68]

Epstein-Barr virus does not have a specific thymidine kinase, so reports of its sensitivity to acyclovir, albeit at high drug concentrations, were surprising.[49,70-72] It appears that Epstein-Barr DNA polymerase is acutely sensitive to the triphosphate of

acyclovir[72] and the small amount generated by other enzymes is enough to cause inhibition. Cytomegalovirus DNA polymerase probably has a similar enhanced sensitivity.[46,52,73,74]

Acyclovir was shown to be well distributed throughout all tissues, including the brain, in several species of animals[43,75-80] with a plasma half life of approximately two and one half hours. Administration of radiolabelled acyclovir showed that only small amounts were metabolised, ~95% of the dose was recovered in the urine. The only metabolites so far reported are 9-[(carboxymethoxy)methyl]guanine (25) and 9-[(2-hydroxyethoxy)methyl]-8-hydroxyguanine (26), with the amounts depending on the species.[76,77,80]



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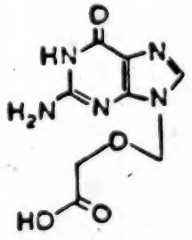


(25)

Therapeutic effects have been demonstrated for a variety of viral infections in different animal models, including herpes encephalitis in mice,[43,81-83] keratitis in rabbits,[43,84-93] cutaneous and genital herpes in mice and guinea pigs,[43,81,94-97] herpes B in rabbits,[51] simian-varicella virus in African green monkeys[98,99] and cytomegalovirus in mice.[100,101]

In primary viral infections the nerve ganglia are invaded and it is believed that it is here that the latent virus resides until reactivated.[102] Acyclovir has been shown to give total, partial or no protection against the establishment of latency in primary infections depending on the time after infection therapy was commenced.[49,89,102-110] Unfortunately once latency was established, treatment with acyclovir had no effect on the number of latent genomes,[103-108] although Pavan-Langston et al. have claimed to observe a decrease.[111,112] Even though the latency could not be prevented, clinical reactivation could be inhibited by treatment before and after the reactivating event.[110,113]

A problem with the continued use of a particular drug is the development of resistance. In principle, resistance to acyclovir could occur at one of two loci, either at the DNA polymerase or the thymidine kinase

level. It has been shown[114-122] that passage of herpes virus in the presence of acyclovir gives a relatively high frequency of drug resistant mutants. Virtually all the drug resistant strains do not have the ability to phosphorylate acyclovir. However there have been four examples of mutants at the polymerase level.[67,116-118,123] Crumpacker[123] isolated the polymerase from one of these mutants and reported that it was poorly inhibited by acyclovir triphosphate. The development of resistance with clinical herpes isolates in human cell lines[121,124] and in clinical trials[125-127] has been noted recently. This may not prove to be a problem since the majority of clinical cases result from reactivation of latent virus. It has been shown that thymidine kinase deficient mutants of HSV-I are nine hundred times less virulent than the wild type.[114,128]

Preclinical toxicology studies using acyclovir in animal and <u>in vitro</u> models showed a low order of toxicity.[84,129-131] Secondary toxicity is seen at higher doses due to the precipitation of acyclovir in the nephrons of the kidney, causing transient impairment of renal function. Acyclovir was considered safe for clinical trials and a report appeared describing the successful treatment of epithelial herpetic

keratitis.[132] Much work has since been published on the successful treatment of ocular infections[87,133-134] showing acyclovir to be at least as effective[135-138] as, or more effective[139-142] than idoxuridine (18), vidarabine (20) or trifluoromethyl-2'-deoxyuridine (19). An irritation due to the ointment base was observed which did not warrant withdrawal of treatment.[143]

One third of the general population suffer from recurrent herpes infections of the lip or perioral area, but for the most part these infections are merely a nuisance. However, in immunocompromised patients the infections are much more severe, even becoming life threatening.[144] Thus there have been many reports of the use of acyclovir, both prophylactically and chemotherapeutically, in such cases.[145] As noticed *in vitro*, the susceptibility of the viruses decrease in the order HSV;[146-155] VZV;[155,156] EBV;[158] and CMV.[159-162]

Primary genital herpes infections have responded well to treatment with acyclovir whether the administration was topical,[163,164] oral[165,166] or intravenous.[167] However, in recurrent infections slight or no clinical benefit was observed.[163] This is likely due to the fact that these episodes are of short

duration with natural healing occurring one or two days after the onset, thus making any effect of acyclovir treatment difficult to observe. It has been suggested[168] that since two-thirds of sufferers of genital herpes experience prodromes, patient initiated therapy may be more rewarding.

Treatment of herpes infections with acyclovir in combination with other drugs has been reported with synergistic and additive effects having been seen depending on the infection and the other compound.[169-172]

Acyclovir pharmacokinetics in humans were very similar to those observed in animals, with desirable plasma levels of the drug readily obtainable by either oral[173] or intravenous administration[174-180] and with a half life of approximately three hours in patients with normal renal function.[176,181-183] Most of the dose was recovered in the urine[184-186] with only metabolites (25) and (26) being observed in small amounts.[177,187] Metabolic removal accounted for a higher percentage in patients with some type of renal failure[185,188] as would be expected.

Finally, as observed in animals, the major toxicity noted has been that of renal dysfunction[189,190] which appears after high dose bolus injections. However this can be circumvented if the drug is administered by slow

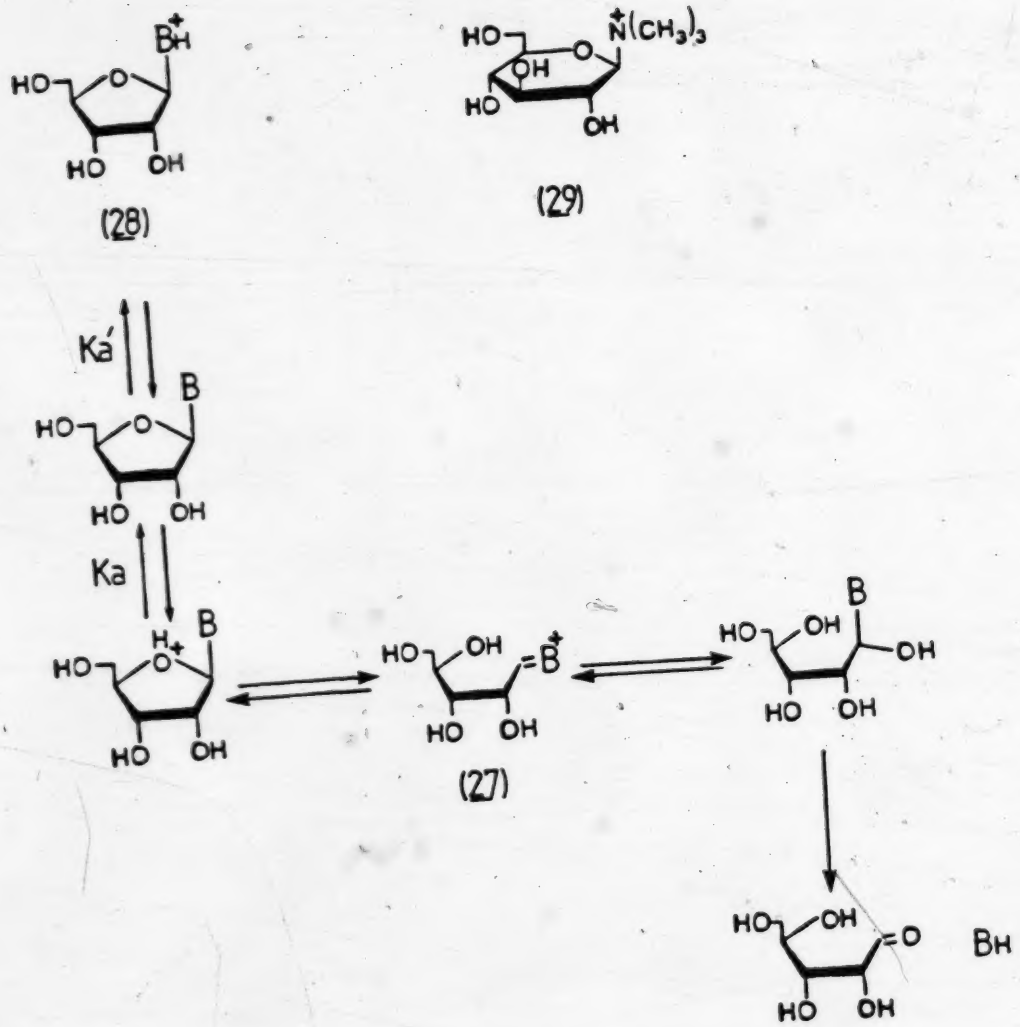
infusion with adequate hydration.[190]

Acid Hydrolysis of Nucleosides

The mechanism of acid catalysed hydrolysis of nucleosides has been of considerable interest since the report of the release of heterocyclic bases from nucleic acid in 1891.[191] The impetus for the earlier investigations was the challenge to elucidate the structure and sequences of nucleic acids. It was known that mild hydrolysis produced apurinic acids,[192-194] indicating the greater lability of the purine glycosyl bond. More vigorous conditions hydrolysed the sugar-phosphate backbone linkages giving rise mainly to pyrimidine nucleoside diphosphates, an observation that led Levene to propose the alternating pyrimidine-purine theory.[195-200] However, it was shown later that pyrimidine diphosphates could survive the acidic conditions employed.[201-203]

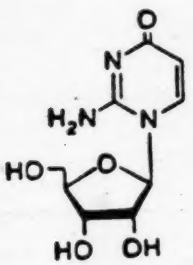
The first published mechanism for the hydrolysis of nucleosides was proposed by Kenner[204] and involved a Schiff base intermediate (27), based on the accepted mechanism for glycosylamine hydrolylsis (Scheme 1).[205,206] Kenner proposed that protonation would occur first on the more basic purine, but postulated that this species (28) would be stable. It

Scheme I



was reported that the quaternary ammonium salt (29) was quite stable.[207]

Dekker later supported this mechanism but stressed the importance of a nitrogen atom being in the proximity of the sugar ether oxygen, thus facilitating the intramolecular transfer of a proton.[208] Dekker suggested that this rationalised why (6), (30) and (31) hydrolysed more rapidly than the naturally occurring pyrimidine nucleosides.



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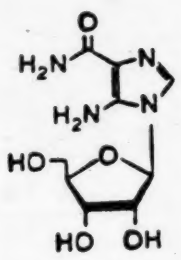

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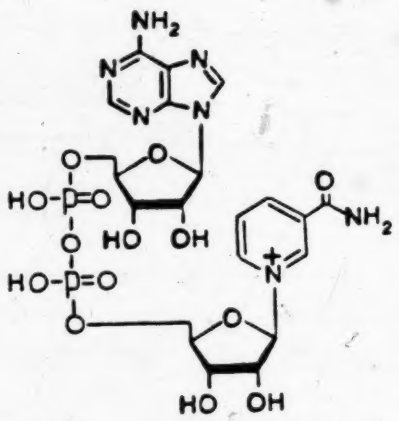

(32)

Alivisatos[209,210] showed that of a variety of imidazole ribonucleotides only (31) was hydrolysed

under conditions of the Orcinol test.* This finding agreed with Dekker's proposal, but the authors also found both glycosyl bonds in diphosphopyridine nucleotide (32) to be labile. This observation was clearly incompatible with a Schiff base mechanism but was ignored by other authors.

Established characteristics of such a Schiff base mechanism are general acid catalysis, rapid anomerisation and bell shaped pH/rate profiles.[206] Early detailed kinetic investigations on both purine[211,212] and pyrimidine nucleosides[213-216] showed no such phenomena. Most authors still explained their results in terms of the then accepted mechanism (Scheme I).

If formation of the Schiff base were the rate determining step as proposed, then electron withdrawing groups in the base moiety should retard the rate of hydrolysis. It was found that such electronegative groups enhanced the cleavage in both pyrimidine[215-221] and purine nucleosides.[222-225] Such inconsistencies led Shapiro[217,218] to propose an alternative mechanism involving an oxocarbonium ion (33) intermediate

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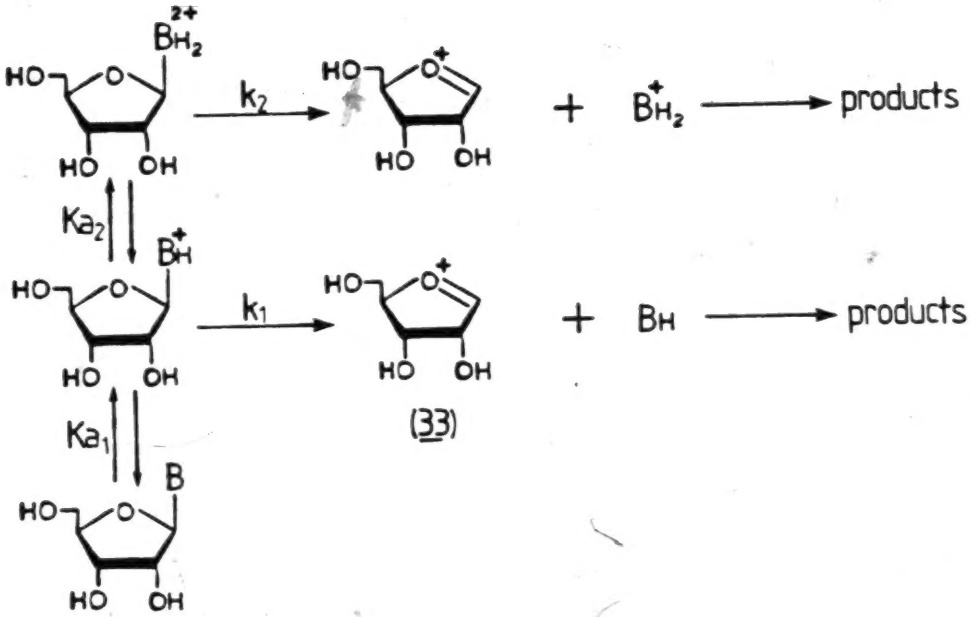
* 6 molar HCl, 100°C for 20 minutes.

Scheme II



(33)

(Scheme II).* Effects resulting from the basicity of the departing heterocycle and pH/rate profiles were consistent with such a mechanism, as were observations in most of the earlier work.

Zoltewicz reported the pH/rate profiles of some purine nucleosides and observed a linear dependance even at the pKa of the nucleosides.[228,229] This linearity was rationalised to result from the hydrolysis of both mono and diprotonated species (Scheme II). Repetition of some of this work by others led to the observation of slight curvatures.[230] Substitutions in the purine ring that change both the pKa and acid catalysed hydrolysis rate values made the expected curvature more obvious.[224] Solvent participation in the rate determining step was ruled out by: comparison of N-7 and N-9 isomers,[231] theoretical considerations,[232] Bunnett values,[218,229,230] activation entropy values[222,228,229,231] and by an observed secondary α-deuterium isotope effect.[233]

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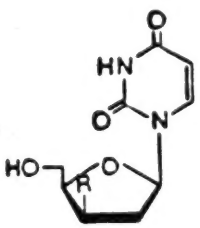
* The stability of such an intermediate is a matter of debate with some authors calculating a lifetime of 10^{-11} to 10^{-15} secs,[226] whilst others compare it to the diphenylmethyl carbonium ion.[227]

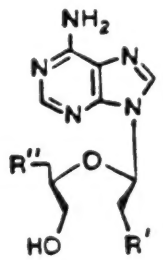
Lonneberg reported the effect of various metal ions in hydrolysis solutions[234-237] under conditions in which it is known that co-ordination of the metal to the heterocyclic base occurs.[238-240] With the exception of mercury,[236,237] all metal ions had a rate retarding effect. This was rationalised by assuming competition by metal ions for the sites of protonation.

Whether the hydrolysis of nucleosides occurs via a Schiff base or oxocarbonium ion intermediate, electronegative groups in the sugar moiety would be expected to destabilise both types of intermediates and thus retard the rate of hydrolysis. This has been observed; 2'-deoxynucleosides hydrolyse faster than their ribocounterparts,[193,241-243] as do 3'-deoxy-nucleosides,[222] and 2',3'-dideoxynucleosides are the most labile.[222] The effect of these hydroxyl functions may not be limited to their electronegativity. Garrett suggested[222] that the 2'-hydroxyl function might compete as a proton acceptor site, or that hydrogen bonding to the base might be important.[222,244] Methylation of these hydroxyls did cause an increase in stability.[245-247] However, this may be due to inter-ference with solvation of the developing carbonium ion. The configuration of the 2' and 3' hydroxyl

groups had a significant effect,[222,248] which was proposed to be due to hydrogen bonding. It is likely that this would cause a change in conformational energy in the transition state. A definite indication that electronegativity was not the only factor was illustrated by the decreasing order of hydrolytic stability of (34), (35) and (36).[249] Even though more



(34) R = Cl
(35) R = F
(36) R = OH

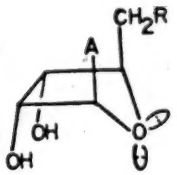


(37)

than one factor is operating, the protection of nucleoside hydroxyls with electron withdrawing ester blocking groups has allowed the synthesis of some acid sensitive compounds.[250-256] It was suggested[254] that aromatic ester groups could stack with the heterocyclic aromatic base and thereby alter its' reactivity. This was shown not to be significant in terms of the acid catalysed hydrolysis stabilities of adenine

nucleosides.[256]

To eliminate conformational factors, Robins and Cross[257] studied effects of electronegative groups in a series of "oxidised-reduced" acyclic adenine nucleosides (37). Correlations between electronegativity and rates of hydrolysis were observed, with 2'-substituents having the largest effects. 5'-Substituents exhibited the same trend, presumably by decreasing the availability of the ring oxygen's lone electron pairs. Similar effects have been observed in the hydrolysis of glycopyranosides.[258] The most striking feature of the Cross study[257] was that the ring opened nucleosides hydrolysed at a faster rate than their cyclic counterparts. This can be explained by considering the conformation that will allow an antiperiplanar relationship between the ring oxygen's lone electron pairs and the departing heterocycle, namely ${}_{O}E$ (01' exo P ≅ 270) (38).[259] Due to 1,3 pseudo diaxial and eclipsing interactions this conformation would be expected to be very strained. In contrast the acyclic oxidised-reduced nucleosides can achieve this antiperiplanar relationship quite easily (39), thus facilitating hydrolysis. This hypothesis is supported further by the observations[260,261] that purine nucleoside 3',5'-cyclic phosphates (40) are much more



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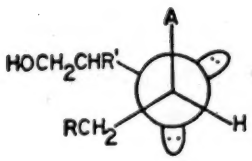

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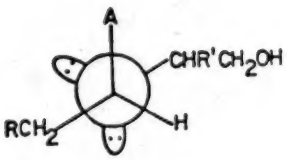
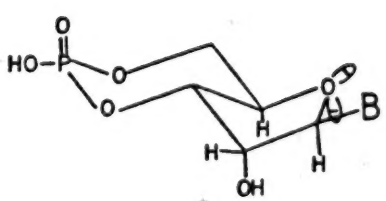


(39b)

resistant to hydrolysis than the corresponding acyclic nucleotides. In the trans fused bicyclic system of (40) the $_{O}E$ conformation cannot be realised. The transient appearance of 3' and 5' nucleotides[260,261] and observation of similar rates for the liberation of



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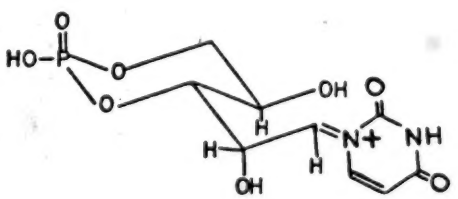
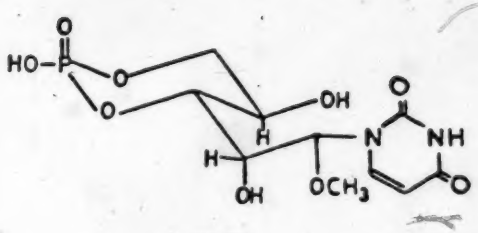


(41)

purine bases and hydrolysis of the cyclic phosphate ring strongly imply that glycosyl bond hydrolysis proceeds only after the cyclic phosphate has been opened. With uridine and thymidine cyclic phosphates, a dramatic increase in rate was observed.[260-262] This suggests that these two pyrimidine nucleosides do not require the $_{o}E$ conformation for facile hydrolysis. On the basis of the Schiff base mechanism (Scheme I), in the intermediate (41) ring strain is relieved and could account for such an increase in rate. In contrast, cytidine 3',5'-cyclic phosphate was quite stable under such conditions and only the cyclic phosphate ring was hydrolysed.[260,261] These data suggest that purine and cytosine nucleosides have only the oxocarbonium pathway (Scheme II) available to them, whereas uracil-type nucleosides may hydrolysed via both this and the Schiff base mechanism. Further observations are consistent with this assumption. Robins and MacCoss[260] found that treatment of uridine 3',5'-cyclic phosphate in anhydrous methanolic hydrogen chloride led to the isolation of (42). Similarly the octosyl acid (43) was shown to give such an intermediate which was isolated as the triacetate[263] (44).



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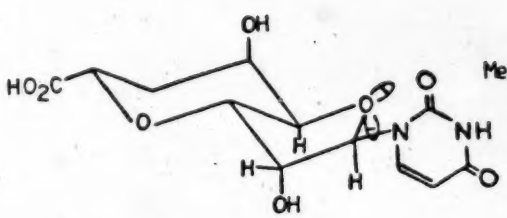

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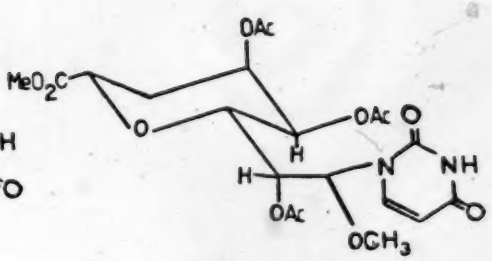
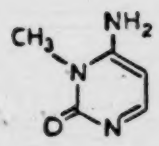


(44)

In aqueous solution no analogous hemiacetal intermediates were detected, but Cadet reported the isolation of small amounts of pyrimidine α-furanosides, and β and α-pyranosides from the partial hydrolysis of 2'-deoxyuridine and thymidine. These products would be expected from a Schiff base mechanism. 2'-Deoxycytidine and 5-bromo-2'-deoxyuridine did not give such products.[264]

Previous work[218,221,261,262,264,265] indicates that cytidine nucleosides behave differently from uridine and thymidine nucleosides. The most obvious difference between these heterocycles is the ease of protonation: cytosine pKa = 4.6,[266] uracil and thymine pKa = -3.4.[218] It seems reasonable that cytosine nucleosides protonate at the N-3 position on the aromatic heterocycle and then hydrolyse, like purine nucleosides, via an oxocarbonium ion intermediate (Scheme II).[217,218,221] The rate of hydrolysis of a cytosine nucleoside is slower than that of the corresponding purine nucleoside.[218] This can be explained, apart from the fact that purine nucleosides are known to diprotonate at lower pH, by considering the basicity of the departing heterocycle. Previous studies have shown a good correlation between the rate of hydrolysis and the basicity of the leaving nitrogen atom.[215-224,227,267,268] 3-Methylcytosine (45)

(pKa = 7.38[269]) and 7-methylguanine (46) (pKa = 3.5[266]) are good models for the leaving groups* during the acid hydrolysis of cytidine and guanosine, respectively.



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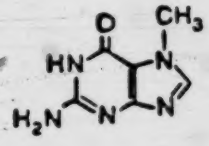

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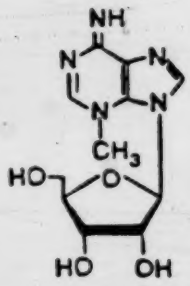

(47)

The basicity of the leaving group is probably an important factor in determining rates of hydrolysis of N-7[223,231,272,273] and N-3[274] ribosyl purines. However, if only small differences in basicity are found between two leaving groups, then other factors such as site of protonation or steric strain might be more important. The introduction of strain via a "peri" located alkyl group, for example in 3-methyladenosine

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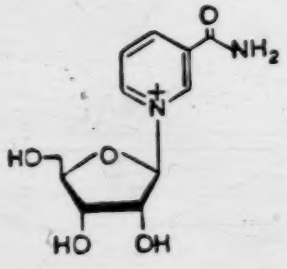
* Protonation of cytidine and guanosine is known to occur at N-3[270] and N-7,[271] respectively.

(47), has caused large rate enhancements.[275-281] Alkylations of other sites of the heterocycle had lesser effects.[222,228,282]

From data presently published it seems that both the Schiff base mechanism (Scheme I) and the oxocarbonium ion mechanism (Scheme II) can function depending on the structure of the nucleoside. If a general scheme to account for the hydrolysis of all nucleosides is desired, then both pathways have to be included (Scheme III). Which pathway is more energetically favourable for a particular nucleoside depends on its structure. Thus, pyridine nucleosides (48) can hydrolyse only via a pH independent oxocarbonium ion pathway (k_{lo} in Scheme III),[227,268,283,284] benzimidazole nucleosides (49) can protonate only once and so



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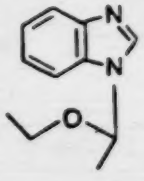
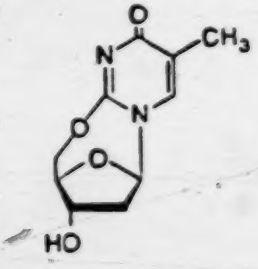
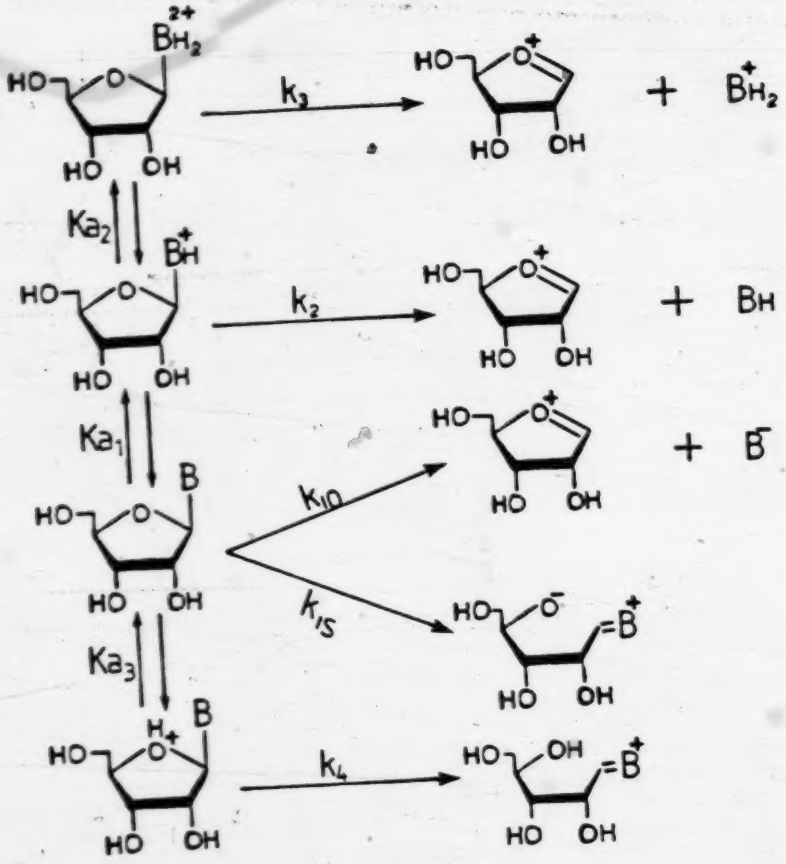


(49)

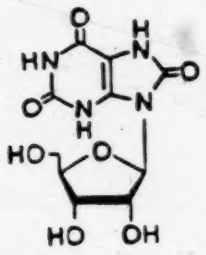


(50)

Scheme III



the diprotonated pathway (k_3) is not available in contrast to purine nucleosides.[224,228-230] The cyclonucleoside (50) is much more labile than thymidine (13)[285] due to the availability of the protonated monocation route (k_2). Least is known about nucleosides such as thymidine (13), uridine (9) and 9-(β-D-ribofuranosyl)uric acid (51) which are protonated only at very low pH values (<0). It is reasonable to assume



(51)

that the sugar can become a competitive site of protonation* in the hydrolysis of these nucleosides

---

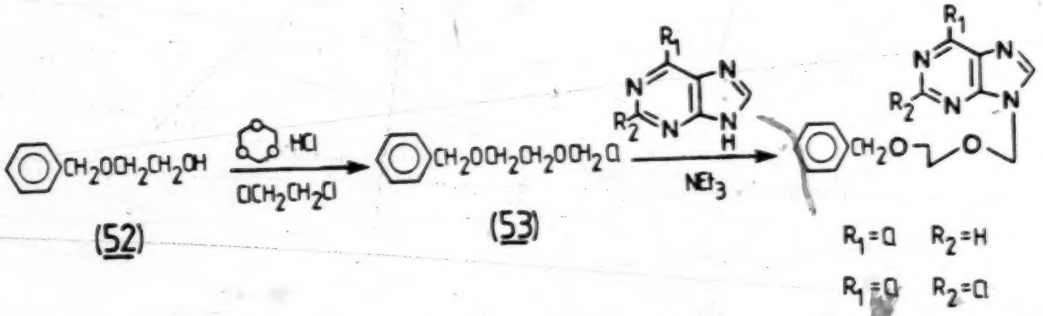
* Estimated from tetrahydrofuran pKa = -2.1.[286]

and a Schiff base mechanism is more likely. Shapiro noted[217] that deoxyuridine and thymidine underwent a slow unimolecular cleavage at neutral pH, but whether this occurs via a k_{1o} or k_{1s} pathway (Scheme III) or a combination of both is not known. Garrett[219] reported that 5-substituted-2'-deoxyuridines hydrolysed faster than the parent nucleosides whether the substituent was electron donating or withdrawing. This is consistent with a shift between two types of mechanism.

RESULTS AND DISCUSSION

1. Syntheses

The first syntheses of (2-hydroxyethoxy)methyl nucleoside analogues employed (2-benzyloxyethoxy)-methyl chloride (53) as the alkylating agent.[26,43] Treatment of 2-benzyloxyethanol (52) with paraformaldehyde and hydrogen chloride in dichloroethane gave (53) which decomposed during attempted isolation. Immediate coupling of intermediate (53) with chloropurines gave low yields of the desired N-9 isomers.[26,43,287,288] Higher yielding syntheses have been developed using



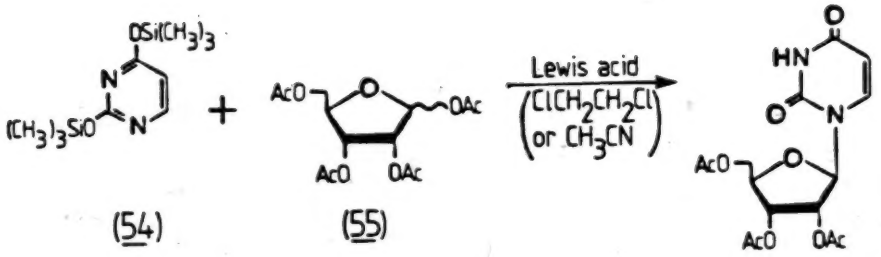
other coupling methods.

Lewis acid catalysed couplings of both silylated purines and pyrimidines with suitably protected sugars

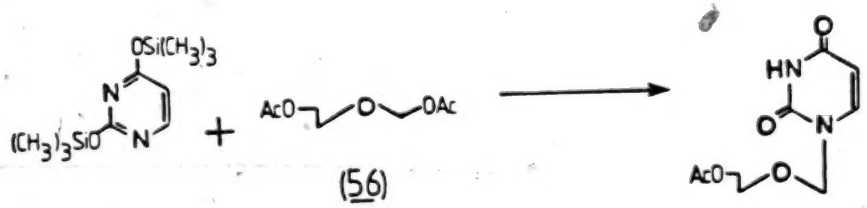
have been reported to occur with high regiospecificity and in good yields.[287,290] An example of this is the coupling of the 2,4-bis-O-trimethylsilylated uracil (54) with tetra-O-acetyl ribose (55). We expected that analogously favourable coupling of (2-acetoxyethoxy)-methylacetate (56) with silylated bases would occur also. Reaction was observed, but the couplings generally occurred in only ~50% yields. This also has been reported by other workers.[291-293] The diminished yields may result from the difference in stability of the proposed intermediate oxocarbonium ions.[290] Clearly, (57) is less stable than (58) and thus it might decompose before coupling with the silylated heterocycle can occur.

Acid catalysed opening of 1,3-dioxolane (59) with acetic anhydride provided a facile synthesis of (56).[294] Treatment of (59) with acetyl chloride or acetyl bromide also resulted in the opening of the dioxolane ring to give good yields of (2-acetoxy-ethoxy)methyl chloride (60) or (2-acetoxyethoxy)methyl bromide (61).

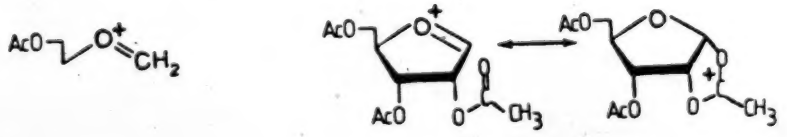
Samples of (60) were consistently contaminated by a tenacious by-product which was not removed completely


OSi(CH3)3
(CH3)3SiO
N
N
AcO
OAc
AcO
OAc
Lewis acid
(ClCH2CH2Cl
or CH3CN)
HN
O
O
N
AcO
AcO
OAc

(54) (55)


OSi(CH3)3
(CH3)3SiO
N
N
AcO
O
OAc
HN
O
O
N
AcO
O

(56)

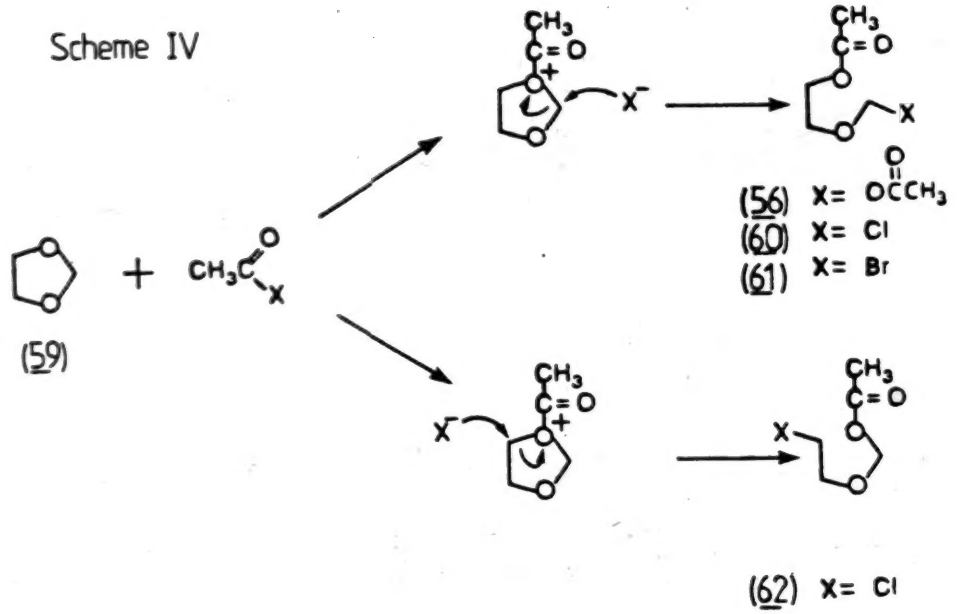

AcO
O+
CH2
AcO
O+
AcO
O
CH3
AcO
O
O
+
CH3

(57) (58)

by repeated fractional distillations. Its' presence was detected most easily by ^{13}C NMR spectroscopy. If the mechanism of the acetal ring opening is as shown in Scheme IV, then attack of chloride ion at position four of the ring would give (2-chloroethoxy)methyl acetate (62), a product which would be expected to have similar physical properties to (60). Compound (62) was synthesised from 2-chloroethanol by treatment with paraformaldehyde and hydrogen chloride followed by sodium acetate in glacial acetic acid. Addition of synthetic (62) to the impure sample of (60) resulted in the appearance of new ^{13}C NMR signals, indicating that (62) was not the original impurity.

This problem was not pursued further since it was found that acetyl bromide opened 1,3-dioxolane (59) cleanly and gave a pure sample of (61) in 88% yield after vacuum distillation. This bromomethyl ether derivative could be stored in the refrigerator without decomposition. There have been recent reports of the opening of (59) with trimethylsilyl iodide to give the highly reactive (2-trimethylsilyloxyethoxy)methyl iodide.[295-297] This reagent requires repetitious generation just before use and low reaction temperatures due to its instability. We found that coupling

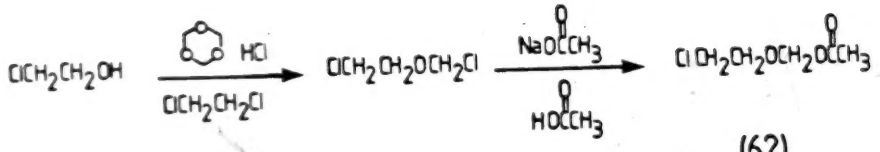
Scheme IV



(59)

(56) X= $OCCH_3$
(60) X= Cl
(61) X= Br

(62) X= Cl



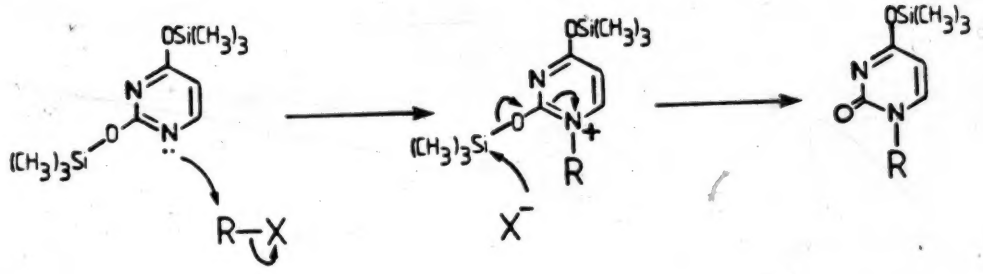
(62)

of (61) or chloromethylmethyl ether (63) with silylated pyrimidines occurred under mild conditions without catalyst using a silyl-modification of the Hilbert-Johnson procedure[298-300] (Scheme V). An excess of silylated heterocycle was employed in order to avoid dialkylation, and the use of low temperature (0°C) resulted in formation of only traces of the isomeric N-3 product. Consistently high yields were obtained (see Table I).

Electrophiles are known to attack the 5-position of a pyrimidine ring[301] and this route was chosen to prepare a series of 1-[(2-hydroxyethoxy)methyl]-5-substituted cytosines (Scheme VI). Since strongly acidic conditions are required for the nitration of cytosine, this was performed prior to coupling with the (2-acetoxyethoxy)methyl side chain. Chlorination and brominaton were performed directly on 1-[(2-hydroxyethoxy)methyl]cytosine (88). Chlorination proceeded very smoothly under the conditions recently described by Ryu and MacCoss.[302] A modification of this procedure produced 5-bromo-1-[(2-hydroxyethoxy)methyl]-cytosine (94), albeit in lower yield (46%).

1-[(2-Acetoxyethoxy)methyl]-2-thiouracil (72) was desulphurised using Raney nickel to give 1-[(2-acetoxy-

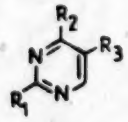
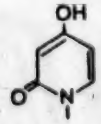
Scheme V



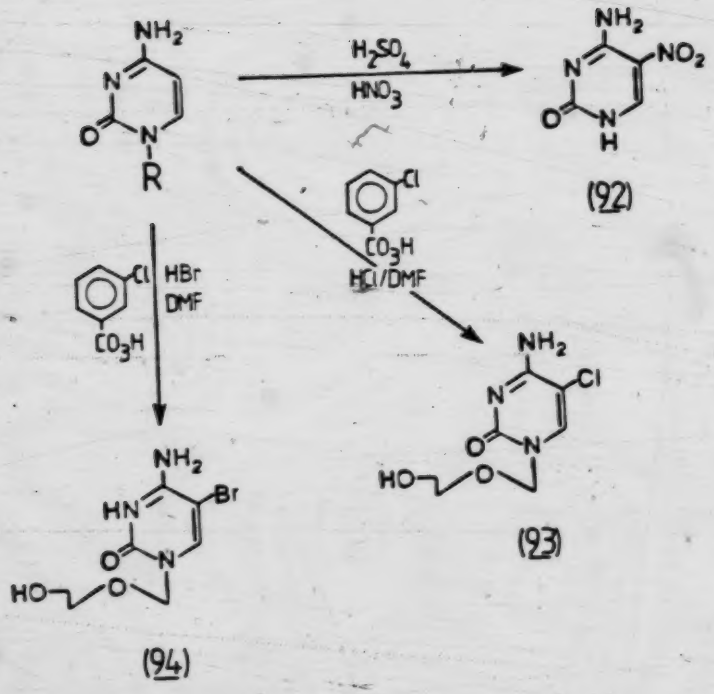
(61) RX= $CH_3\overset{O}{\overset{\|}{C}}OCH_2CH_2OCH_2Br$

(63) RX= CH_3OCH_2Cl

TABLE I. Synthetic Data for 1-[(2-hydroxyethoxy)methyl]pyrimidines.

Heterocycle			Alkylating Agent	Compound (yield %)	Deblocking Method	Compound (yield %)
						
R_1	R_2	R_3				
OH	OH	H	61	64 (79)	A	80 (91)
OH	OH	CH_3	61	65 (84)	B	81 (93)
OH	OH	F	61	66 (84)	B	82 (96)
OH	OH	Cl	61	67 (87)	A	83 (98)
OH	OH	Br	61	68 (86)	A	84 (78)
OH	OH	I	61	69 (79)	B	85 (92)
OH	OH	NO_2	61	70 (86)	B	86 (92)
OH	OH		61	71 (87)	A	87 (91)
SH	OH	H	61	72 (83)		
OH	OH	H	63	73 (86)		
OH	OH	CH_3	63	74 (78)		
OH	OH	NO_2	63	75 (72)		
OH	NH_2	H	61	76 (85)	B	88 (94)
OH	NH_2	NO_2	61	77 (75)	B	89 (91)
NH_2	OH	H	61	78 (87)	A	90 (96)
			61	79 (88)	B	91 (83)

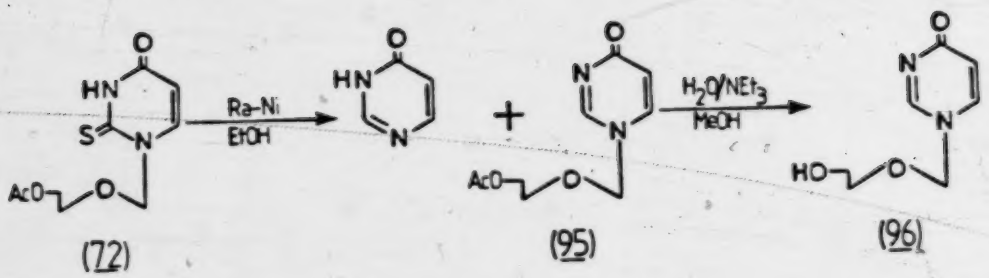
SCHEME VI


NH2
N
O
N
R
H2SO4
HNO3
NO2
H
(92)
Cl
CO3H
HCl/DMF
HBr
DMF
NH2
Cl
HO
(93)
NH2
HN
Br
HO
(94)

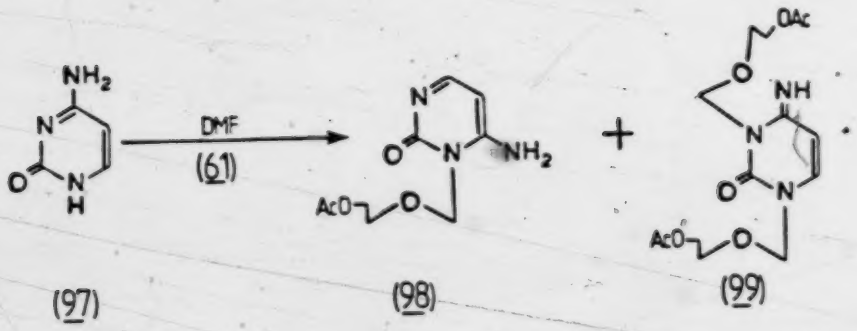
ethoxy)methyl]pyrimidin-4-one (95). In refluxing ethanol this reaction proceeded with concomitant cleavage of the glycosyl bond to give pyrimidin-4-one as the major product. Treatment of (72) at a lower temperature gave a 56% yield of (95) which was deacylated with triethylamine/water/methanol to give the desired 1-[(2-hydroxyethoxy)methyl]pyrimidin-4-one (96).

Coupling of silylated cytosine with (61) occurred almost exclusively at the more basic N-1 position of the heterocycle. It is known that at neutral pH the predominant tautomeric form of cytosine in solution is (97).[303,304] The amidine system (N-3) should be more prone to alkylation than the amide system (N-1) of (97), and this has been observed with methylation.[305,306] Treatment of a suspension of cytosine in dimethylformamide with (61) gave two major products; 3-[(2-acetoxyethoxy)methyl]cytosine (98) and 1,3-bis-[(2-acetoxyethoxy)methyl]cytosine (99) plus traces of the N-1 isomer (76). These products were separated by silica chromatography and deacylated to give 3-[(2-hydroxyethoxy)methyl]cytosine (100) and 1,3-bis-[(2-hydroxyethoxy)methyl]cytosine (101), respectively.

A convenient route for the synthesis of 9-[(2-



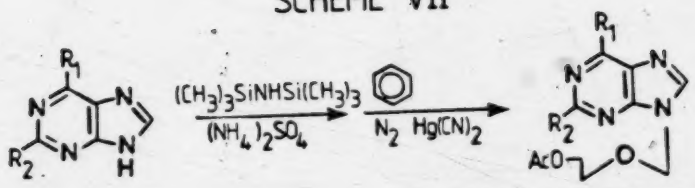
(72) (95) (96)



(97) (98) (99)

hydroxyethoxy)methyl]purines was required also. Chloropurines were chosen as starting materials due to the ease of replacement of the halogen with various nucleophiles.[307] Lee et al.[308] reported that the coupling of a chloropurine and a protected sugar in the presence of mercury (II) cyanide occurred exclusively at the N-9 position. We found that a silylated chloropurine would couple with (61) exclusively at N-9 and in high yield with mercury (II) cyanide as catalyst (Scheme VII). The position(s) of the trimethylsilyl group(s) on the purine are not known, so the exact nature of the intermediate is not defined. Intermediate formation of (105) would explain the regioselectivity, and there is precedent for mercury II binding to the N-7 of purines.[309,310] The coupled products (102), (103) and (104) were transformed further, generally under mild conditions, as shown in Scheme VIII. Due to the significant differences relative to the natural substrate structure, adenosine deaminase converted 2,6-diamino-9-[(2-hydroxyethoxy)-methyl]purine (107) and 2-amino-6-chloro-9-[(2-hydroxy-ethoxy)methyl]purine (108) to acycloguanosine (22) very slowly. This slow rate made syntheses practical on a small scale only. For preparations of larger amounts

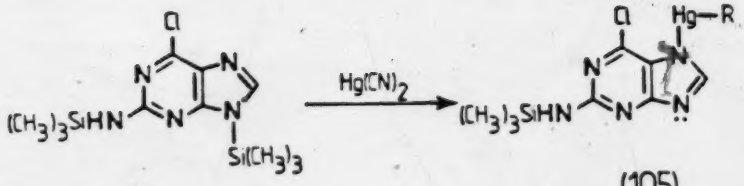
SCHEME VII



(102) $R_1 = Cl$ $R_2 = H$

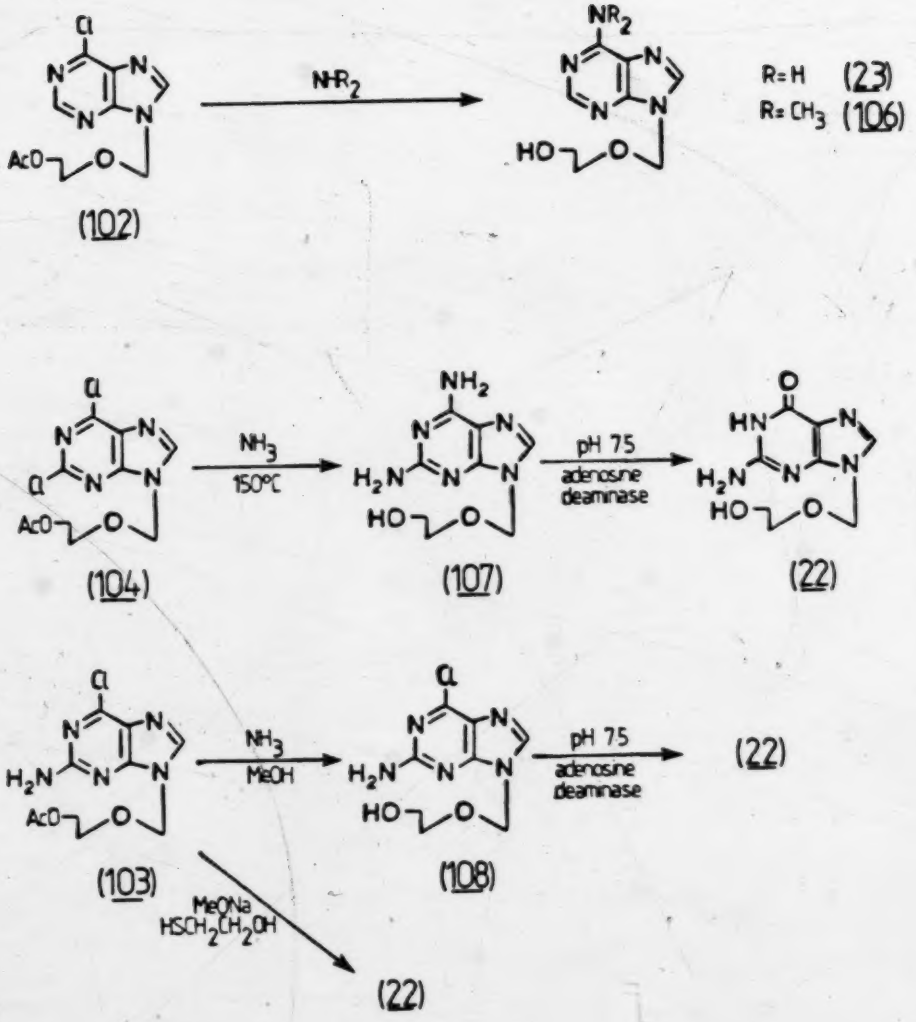
(103) $R_1 = Cl$ $R_2 = NH_2$

(104) $R_1 = Cl$ $R_2 = Cl$



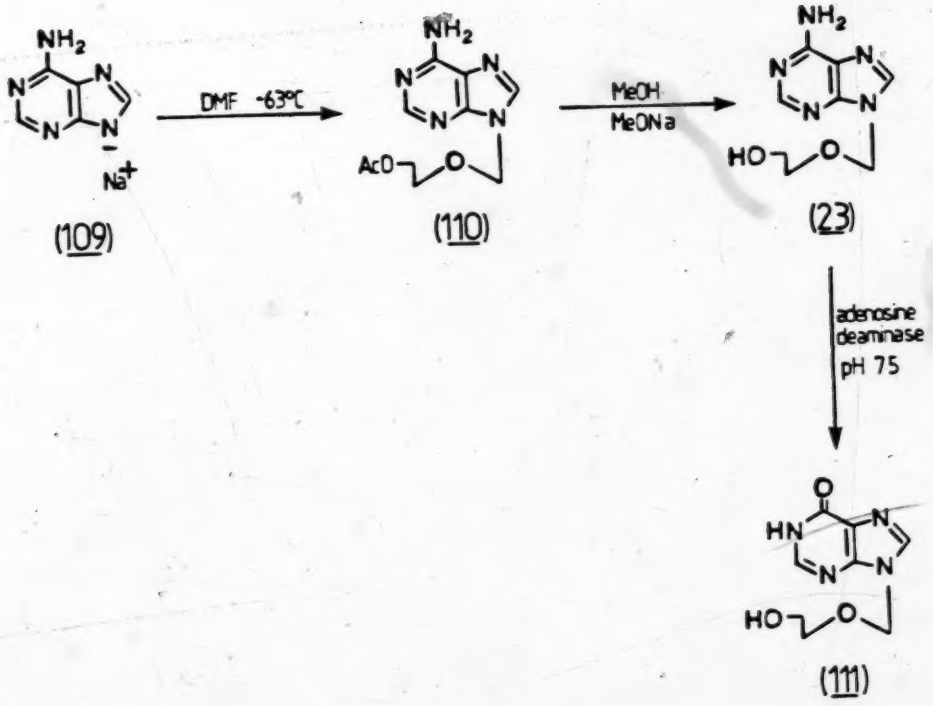
(105)

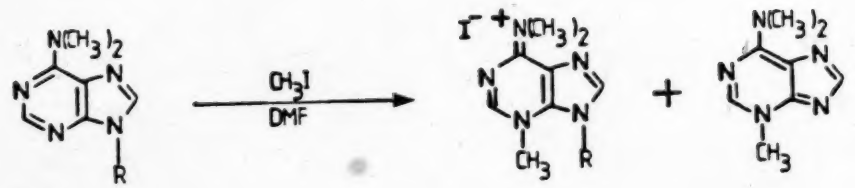
SCHEME VIII


Cl
NHR2
AcO
O
HO
R=H (23)
R=CH3 (106)
(102)
NH3
150°C
NH2
pH 7.5
adenosine deaminase
O
HN
(104)
(107)
(22)
MeOH
(103)
(108)
MeONa
HSCH2CH2OH

of (22), treatment of (103) with sodium methoxide and mercaptoethanol in methanol at reflux was employed.[311] Treatment of (102) with methanolic ammonia gave 9-[(2-hydroxyethoxy)methyl]adenine (23) cleanly, but a more direct synthesis was accomplished by reaction of the sodium salt of adenine (109) with (61) in dimethylformamide at low temperature followed by deacylation of the resulting 9-[(2-acetoxyethoxy)-methyl]adenine (110) with sodium methoxide in methanol. Enzymatic deaminaton of (23) was utilised in the preparation of 9-[(2-hydroxyethoxy)methyl]hypoxanthine (111).

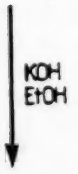
Methylation of the aglycon in adenosine nucleosides is known to occur at the N-1 position.[312] However, introduction of a bulky group at the 6-position may prevent this due to steric repulsion. It has been observed that N^6,N^6-dimethyl-2',3',5'-tri-O-benzoyladenosine (112) methylates at the N-3 position.[313] Similar methylation of 9-[(2-hydroxy-ethoxy)methyl]-N^6,N^6-dimethyladenine (106) was attempted. The desired methylation did occur but with accompanying glycosyl cleavage to give N^3,N^6,N^6-tri-methyladenine (114). This problem was circumvented by allowing the reaction to stand at 0°C for 3 weeks. The

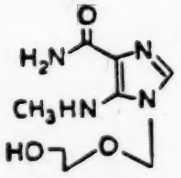

NH2
DMF -63°C
MeOH
MeONa
AcO
HO
adenosine
deaminase
pH 7.5
HN
(109)
(110)
(23)
(111)



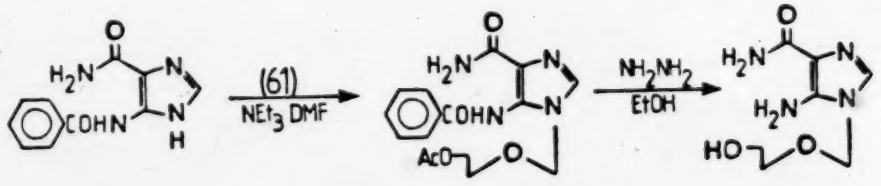
(114)

R= tri-O-benzoylribose (112)
R= $CH_2OCH_2CH_2OH$ (106)





(115)



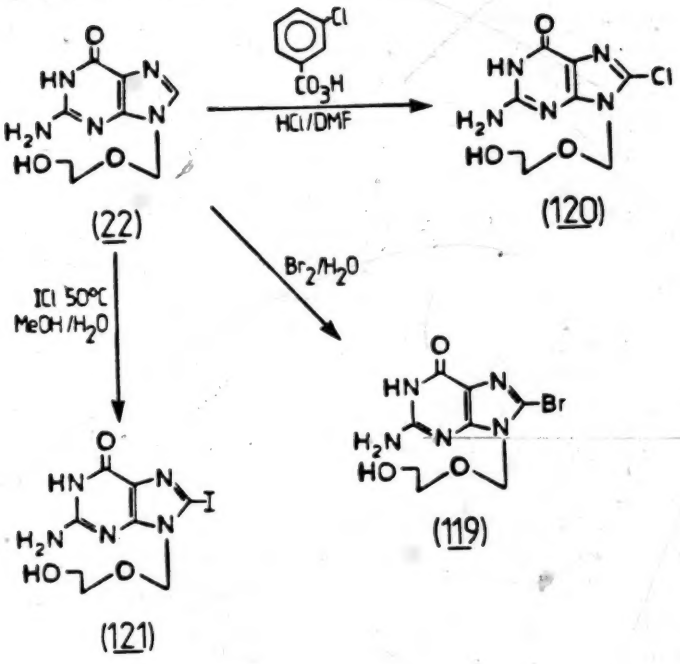
(116) (117) (118)

desired product, 9-[(2-hydroxyethoxy)methyl]-N^3,N^6,N^6-trimethyladenine iodide (113) crystallised from the reaction mixture in 55% yield. Refluxing (113) in ethanolic potassium hydroxide resulted in opening of the pyrimidine ring to give 4-carbamoyl-1-[(2-hydroxyethoxy)methyl]-5-methylaminoimidazole (115). Treatment of 4-carbamoyl-5-benzamidoimidazole (116) with (61) and triethylamine in dimethylformamide gave both N-1 and N-3 isomers.[314] Separation by silica chromatography followed by deprotection of the N-1 isomer with hydrazine in refluxing ethanol furnished pure 5-amino-4-carbamoyl-1-[(2-hydroxyethoxy)methyl]-imidazole (118).

The synthesis of a series of 9-[(2-hydroxyethoxy)-methyl]-8-substituted guanines was desired since the effect of the 8-substituents on viral inhibition and the rate of glycosyl cleavage was of interest. The 8-position of purines is known to be susceptible to electrophilic attack,[307] with bromination occurring readily.[315-318] Bromination of (22) proceeded smoothly in bromine water giving a good yield (83%) of 8-bromo-9-[(2-hydroxyethoxy)methyl]guanine (119). Few studies have been conducted on the iodination and chlorination of purines, but a recent chlorination procedure[302]

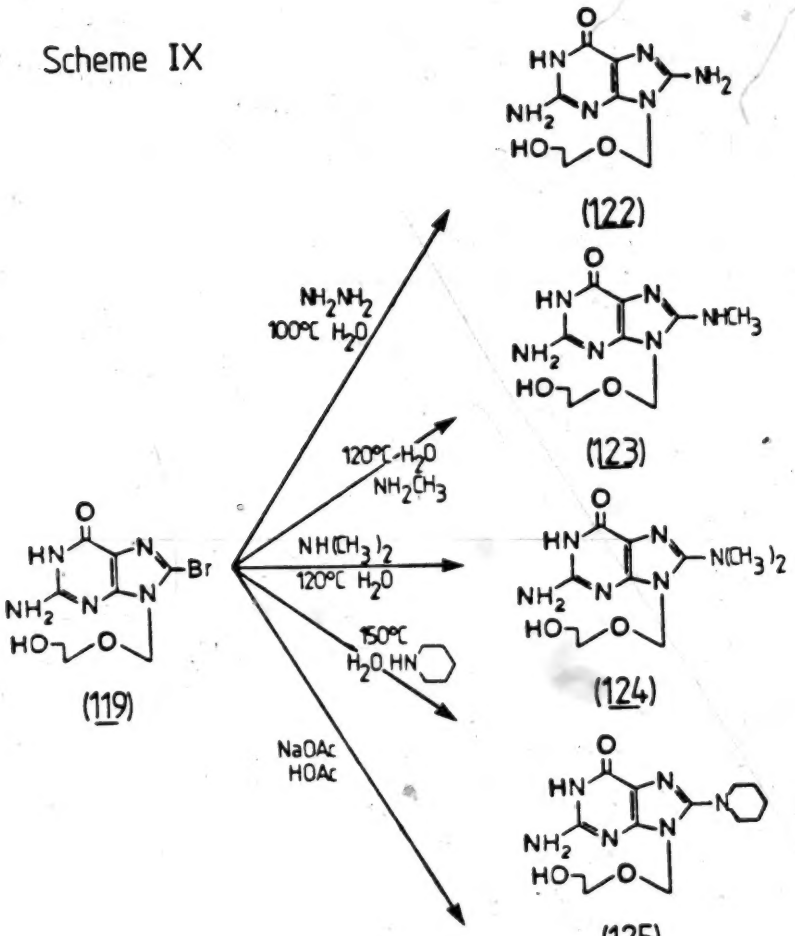
provided an acceptable yield (52%) of 8-chloro-9-[(2-hydroxyethoxy)methyl]guanine (120). The only reported iodination of a guanine nucleoside employed N-iodosuccinamide and n-butyldisulphide in dimethylsulphoxide.[319] This procedure gave poor results with (22). Iodination of (22) was found to proceed readily using iodine monochloride, and a 75% yield of 9-[(2-hydroxyethoxy)methyl]-8-iodoguanine (121) was obtained.

Of the 8-halogenated products, (119), (120) and (121), the 8-bromo derivative (119) was employed for further synthetic transformations due to its' facile synthesis. Nucleophilic replacement of the bromine atom at this position is well known[315-317,320] and most likely proceeds via an addition-elimination reaction mechanism (Scheme X). Several nucleophilic replacements were effected at elevated temperatures (see Scheme IX). Yields were in the range of 67-85%. Purification of products was effected on carbon columns. Treatment of (119) with sodium acetate in glacial acetic acid deserves special mention. In early experiments 8-hydroxy-9-[(2-hydroxyethoxy)methyl]-guanine (126) was obtained directly. However, later experiments consistently gave 9-[(2-acetoxyethoxy)-methyl]-8-hydroxyguanine (127) as the only product.

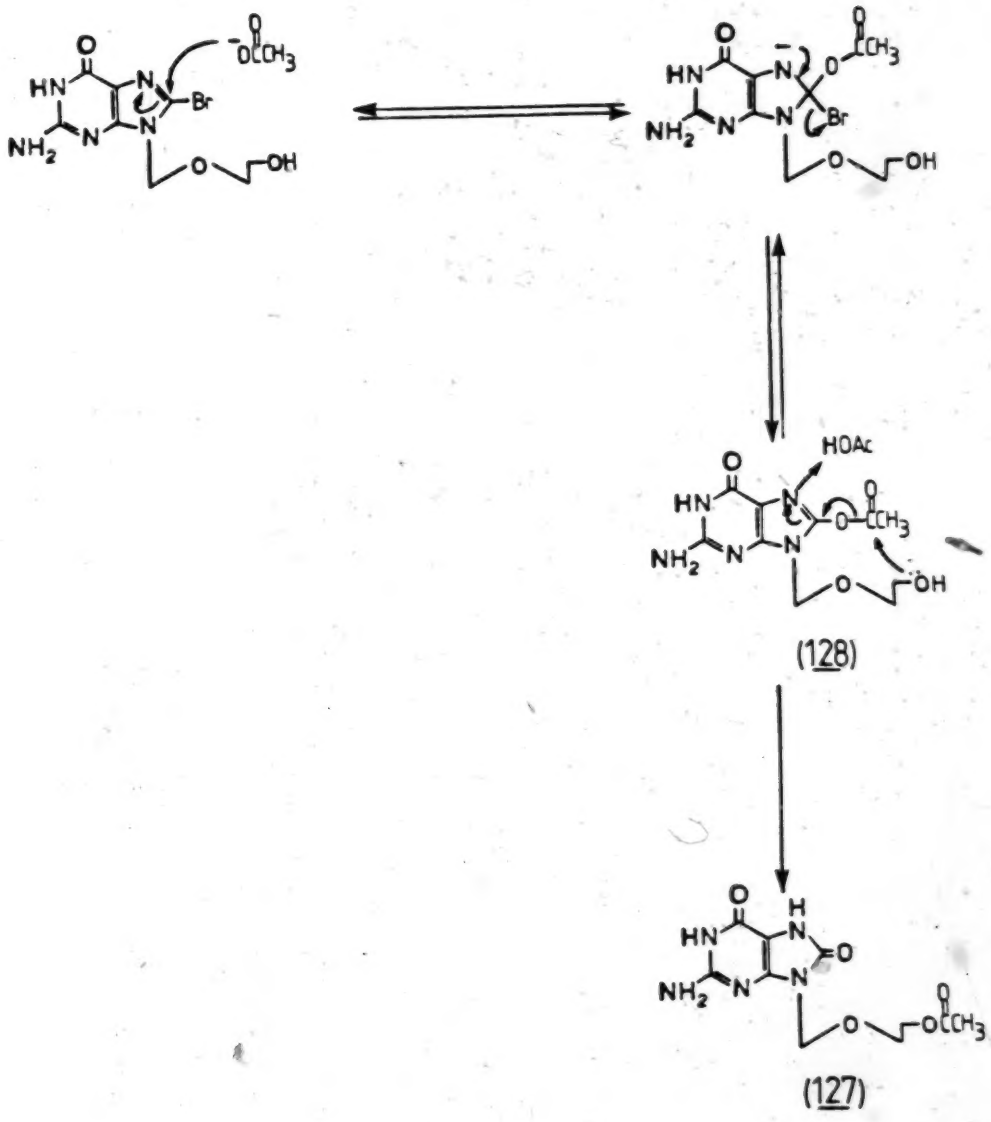


(22) (120) (119) (121)

Scheme IX


NH2NH2
100°C H2O
120°C -H2O
NH2CH3
NH(CH3)2
120°C H2O
150°C
H2O HN
NaOAc
HOAc
Br
(119)
(122)
(123)
(124)
(125)
(127) R = OCCH3
(126) R = H

Scheme X



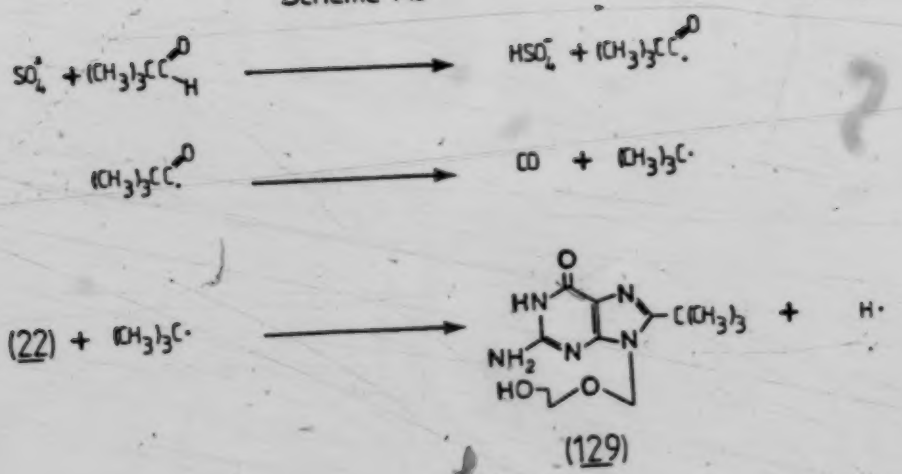
(128)

(127)

These are not normally acylating conditions and a likely explanation of this result is an intramolecular acyl transfer in the intermediate (128) shown in Scheme X. Ikehara[316] did not observe parallel behaviour with the analagous guanosine transformation, but this may be due to the more restricted conformations of the ribose moiety. This occurrence did not present any problems synthetically as (127) could be deacylated with methanolic ammonia to give (126).

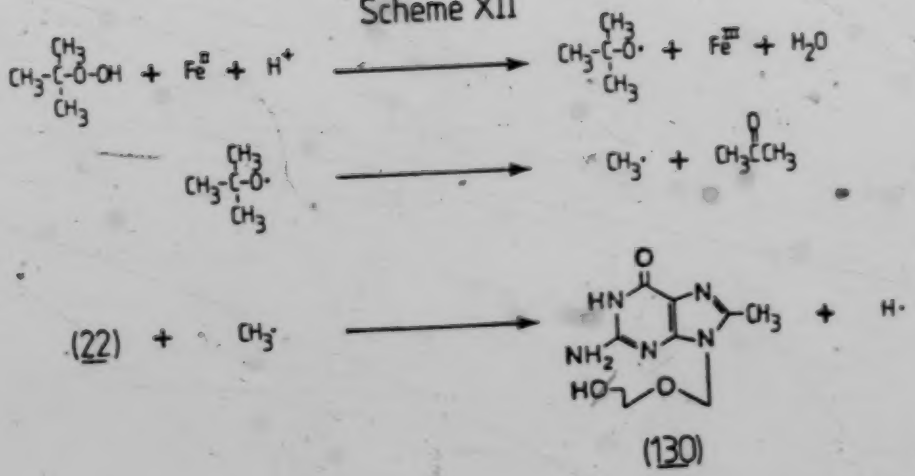
There have been several reports of free radical mediated additions to the 8-position of purines.[321-324] This method of carbon-carbon bond formation was chosen for our study. Tertiary-butyl radicals, generated from pivaldehyde using iron (II) and potassium persulphate, were observed to add to the 8-position of (22) to give a modest yield (42%) of 8-tert-butyl-9-[(2-hydroxyethoxy)methyl]guanine (129) (Scheme XI). The pivalyl radical first formed decomposes to give the tert-butyl radical. A small amount of a by-product which possibly resulted from addition of the acyl radical to (22) was observed but not isolated. Similarly, methyl radicals generated from the homolytic cleavage of tert-butyl peroxide (Scheme XII) were found to add to (22) to give a 64% yield of 9-[(2-hydroxyethoxy)methyl]-8-methylguanine (130).

Scheme XI



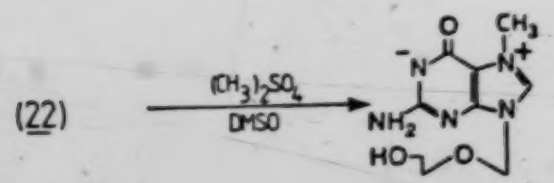
(129)

Scheme XII



(130)

Methylation of guanosine with dimethyl sulphate in dimethyl sulphoxide was known to occur at the N-7 position.[312] This method was employed to produce 9-[(2-hydroxyethoxy)methyl]-7-methylguanine (131). A 56% yield of (131) was obtained, but this betaine could be successfully crystallised only as its hydrochloride salt.



(131)

2. Biological Activities

The biological activities of the (2-hydroxyethoxy)methylnucleosides were determined at the Rega Institute for Medical Research, Katholieke Universiteit, Leuven, Belgium under the supervision of Professor Erik De Clercq.

a) Antiviral Activity

The antiviral properties of the (2-hydroxyethoxy)methyl nucleosides were evaluated in primary rabbit kidney (PRK) cell cultures infected with herpes simplex virus type I (HSV-I), type II (HSV-II), vaccina virus (VV) or vesicular stomatitis virus (VSV). These assay systems have proven useful in previous studies on the comparative efficacy of anti-herpes agents.[45] The reference compounds, (E)-5-(2-bromovinyl)-2'-deoxyuridine (BVDU), 5-iodo-2'-deoxyuridine (IDU) and 9-[(2-hydroxyethoxy)methyl]guanine (acyclovir) had activity levels in accord with those reported previously.[45]

The [(2-hydroxyethoxy)methyl]pyrimidine nucleosides were devoid of activity even at a concentration of 400 µg/ml (Table II). Similar results have been reported recently.[325,326] The inactivity of (E)-5-(2-bromovinyl)-1-[(2-hydroxyethoxy)methyl]uracil (87) contrasts sharply with the potent anti-herpetic activities of its structural "parents", acyclovir and BDVU, from which were derived the (2-hydroxyethoxy)methyl and (E)-5-(2-bromovinyl)uracil moieties, respectively.

With the exception of (106) and (111), all of the [(2-hydroxyethoxy)methyl]purines inhibited HSV replication, albeit at higher concentrations than acyclovir. The introduction of a substituent at the 8-position of

acyclovir was tolerated amazingly well, although generally the activity of the compounds diminished as the size of this group was increased (Table III). The activity of 9-[(2-hydroxyethoxy)methyl]-2-amino-6-chloropurine (108) may be derived from its ultimate transformation to acyclovir by adenosine deaminase.[327] The activity of (108) was found to vary considerably in different cell lines (Table IV), which may be due to the different adenosine deaminase activities. However, the antiviral activity of acyclovir was found to be dependent on the cell line also.

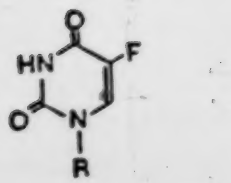
b) Inhibition of Growth of Murine Leukemia L1210 Cells

Although acyclovir is claimed to be essentially non toxic to uninfected host cells,[42-44] it was inhibitory to the growth of murine leukemia L1210 cells at moderate concentrations. This was observed for the other [(2-hydroxyethoxy)methyl]purines with the exception of the inosine analog (111) (Table V). The [(2-hydroxyethoxy)methyl]pyrimidines exerted little if any cytotoxicity toward L1210 cells even at a concentration of 1 mg/ml.

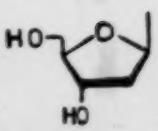
The inhibition of incorporation of 2'-deoxyuridine into DNA relative to that of thymine (Table V) may be taken to reflect the potency by which nucleoside

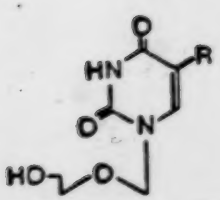
analogues inhibit thymidylate synthetase.[328] It is doubtful if the 9-[(2-hydroxyethoxy)methyl]purines owe any of their cell growth-inhibitory potency to an inhibitory effect on thymidylate synthetase, since their IC_{50} values for L1210 cell growth were far below the IC_{50} for 2'-deoxyuridine incorporation This trend was particularly striking for compound (23).

While not active themselves as inhibitors of tumor cell growth, [(2-hydroxyethoxy)methyl]pyrimidines may potentiate the antitumor activity of 5-fluoro-2'-deoxyuridine and its cogeners. The 1-[(2-hydroxyethoxy)methyl]-5-substituted uracils, (80), (81), (132) and (133) have been reported to inhibit thymidine and uridine phosphorylases,[329-331] the enzymes responsible for the rapid conversion of 5-fluoro-2'-deoxyuridine (134) to 5-fluorouracil (14).[332-334] It was considered that the antitumor potency of (134) might be enhanced if it were co-administered with a phosphorylase inhibitor. Four of our [(2-hydroxyethoxy)methyl]pyrimidines, (71), (82), (85) and (87) were examined for their effects on the L1210 cell growth inhibiting properties of 5-fluoro-2'-deoxyuridine, 5-trifluoromethyl-2'-deoxyuridine, 5-nitro-2'-deoxyuridylic acid, 5-ethyl-2'-deoxyuridine, (E)-5-(2-bromovinyl)-2'-deoxyuridine, 5-bromo-2'-deoxyuridine



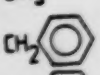
(14) R = H

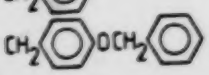
(134) R = 



(80) R = H

(81) R = CH_3

(132) R = 

(133) R = 

and 5-propynyloxy-2'-deoxyuridine. However, the IC_{50} values of these antimetabolites for L1210 cell growth was not significantly altered by the addition of any of the four [(2-hydroxyethoxy)methyl]pyrimidines.

TABLE II. Antiviral activity of 9-[(2-hydroxyethoxy)methyl]pyrimidines in primary rabbit kidney (PRK) cell cultures.

Compound	IC_{50}[a] (μg/ml)			
	HSV-I	HSV-2	VV	VSV
(80) 1-[(2-hydroxyethoxy)methyl]uracil	>400	>400	>400	>400
(82) 5-fluoro-1-[(2-hydroxyethoxy)methyl]uracil	>400	>400	>400	>400
(83) 5-chloro-1-[(2-hydroxyethoxy)methyl]uracil	>400	>400	>400	>400
(84) 5-bromo-1-[(2-hydroxyethoxy)methyl]uracil	>400	>400	>400	>400
(85) 1-[(2-hydroxyethoxy)methyl]-5-iodouracil	>400	>400	>400	>400
(86) 1-[(2-hydroxyethoxy)methyl]-5-nitrouracil	>400	>400	>400	>400
(87) (E)-5-(2-bromovinyl)-1-[(2-hydroxyethoxy)methyl]uracil	>400	>400	>400	>400
(71) (E)-5-(2-bromovinyl)-1-[(2-acetoxyethoxy)methyl]uracil	>400	>400	>400	>400
(91) 1-[(2-hydroxyethoxy)methyl]-3-deazauracil	>400	>400	>400	>400
(81) 1-[(2-hydroxyethoxy)methyl]thymine	70	100	>400	>400
(88) 1-[(2-hydroxyethoxy)methyl]cytosine	>400	>400	>400	>400
(89) 1-[(2-hydroxyethoxy)methyl]-5-nitrocytosine	>400	>400	>400	>400
(90) 1-[(2-hydroxyethoxy)methyl]isocytosine				

[a]Inhibitory $concentration_{50}$ = concentration required to reduce cytopathogenicity of HSV-1 (strain KOS), HSV-2 (strain G), VV or VSV by 50%; HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2; VV, vaccinia virus; VSV, vesicular stomatitis virus.

TABLE III. Antiviral activity of 9-[(2-hydroxyethoxy)methyl]purines in primary rabbit kidney (PRK) cell cultures.

	Compound	IC_{50}[a] (μg/ml) HSV-I	HSV-2	VV	VSV
(22)	9-[(2-hydroxyethoxy)methyl]guanine	0.04	0.04	40	>400
(120)	8-chloro-9-[(2-hydroxyethoxy)methyl]guanine	70	>400	>400	>400
(119)	8-bromo-9-[(2-hydroxyethoxy)methyl]guanine	0.4	0.4	>200	>200(400)[b]
(121)	9-[(2-hydroxyethoxy)methyl]-8-iodoguanine	0.4	0.4	>400	>400
(122)	8-amino-9-[(2-hydroxyethoxy)methyl]guanine	0.7	0.2	>100	>100(200)[b]
(123)	9-[(2-hydroxyethoxy)methyl]-8-methylaminoguanine	7	2	>400	>400
(124)	9-[(2-hydroxyethoxy)methyl]-8-dimethylaminoguanine	20	200	400	>400
(125)	9-[(2-hydroxyethoxy)methyl]-8-piperdylguanine	70	200	>400	>400
(130)	9-[(2-hydroxyethoxy)methyl]-8-methylguanine	0.4	0.4	>400	>400
(126)	8-hydroxy-9-[(2-hydroxyethoxy)methyl]guanine	10	10	>400	>400
(102)	9-[(2-acetoxyethoxy)methyl]-6-chloropurine	70(400)[b]	70	20	>400
(104)	9-[(2-acetoxyethoxy)methyl]-2,6-dichloropurine	2(40)[b]	> 10	> 10	> 10(40)[b]
(103)	9-[(acetoxyethoxy)methyl]-2-amino-6-chloropurine	20(400)[b]	20	70	>400
(108)	2-amino-6-chloro-9-[(2-hydroxyethoxy)methyl]purine	2(400)[b]	20	70	>400
(23)	9-[(2-hydroxyethoxy)methyl]adenine	10	10	20	>400
(106)	9-[(2-hydroxyethoxy)methyl]-N^6 N^6-dimethyladenine	>400	>400	>400	>400
(111)	9-[(2-hydroxyethoxy)methyl]hypoxanthine	>400	>400	>400	>400

[a]Inhibitory concentration$_{50}$ = concentration required to reduce cytopathogenicity of HSV-1 (strain KOS), HSV-2 (strain G), VV or VSV by 50%; HSV-1, herpes simplex virus type 1; HSV-2, herpes simplex virus type 2; VV, vaccinia virus; VSV, vesicular stomatitis virus. [b]In parentheses: minimum cytotoxic concentration causing a microscopically detectable alteration of normal cell morphology.

TABLE IV. Antiviral activity of 9-[(2-hydroxyethoxy)methyl]guanine (22) and 9-[(2-hydroxyethoxy)methyl]-2-amino-6-chloropurine (108) in different cell cultures.

Cell line	IC_{50}[a] (µg/ml)		
	(22)	(108)	Ratio
Primary rabbit kidney (PRK)	0.02	2	100
African green monkey kidney (BS-C-1)	2	40	20
African green monkey kidney (Vero)	2	400	200
Mouse embryo (MO)	0.01	0.3	30
Mouse embryo (BALB/3T3)	0.01	0.4	40
Feline lung (FL)	0.04	7	175
Human cervical carcinoma (HeLa)	0.4	20	50
Human 21-trisomic fibroblast (T-21)	0.07	7	100

[a]Inhibitory $concentration_{50}$ = concentration required to reduce cytopathogenicity of HSV-1 (strain KOS) by 50%. Virus input was 100 $CCID_{50}$ (cell culture infecting $dose_{50}$), adjusted for each particular cell line.

TABLE V. Antimetabolic activity and antiviral index of pyrimidine and purine acyclic nucleosides in primary rabbit kidney (PRK) cell cultures.

Number	Compound	IC_{50}[a] (µg/ml) dThd Incorporation	IC_{50}[a] (µg/ml) dUrd Incorporation	Antiviral index[d]
Pyrimidine acyclic nucleosides				
(80)	1-[(2-hydroxyethoxy)methyl]uracil	>400	>400	-
(81)	1-[(2-hydroxyethoxy)methyl]thymine	>400	>400	-
(82)	5- fluoro-1-[(2-hydroxyethoxy)methyl]uracil	>400	>400	-
(83)	5-chloro-1-[(2-hydroxyethoxy)methyl]uracil	>400	>400	-
(84)	5-bromo-1-[(2-hydroxyethoxy)methyl]uracil	>400	>400	-
(85)	1-[(2-hydroxyethoxy)methyl]-5-iodouracil	>400	>400	-
(86)	1-[(2-hydroxyethoxy)methyl]-5-nitrouracil	>400	>400	>5
(88)	1-[(2-hydroxyethoxy)methyl]cytosine	>400	>400	-
(90)	1-[(2-hydroxyethoxy)methyl]isocytosine	>400	>400	-
(91)	1-[(2-hydroxyethoxy)methyl]-3-deazauracil	>200	>400	-
(87)	(E)-5-(2-bromovinyl)-1-[(2-hydroxyethoxy)methyl]uracil	>200	>400	-
(71)	1-[(2-acetoxyethoxy)methyl]-(E)-5-(2-bromovinyl)uracil			
Purine acyclic nucleosides				
(119)	8-bromo-9-[(2-hydroxyethoxy)methyl]guanine	340	225	450
(121)	9-[(2-hydroxyethoxy)methyl]-8-iodoguanine	>350	>250	625
(122)	8-amino-9-[(2-hydroxyethoxy)methyl]guanine	290	200	667
(123)	9-[(2-hydroxyethoxy)methyl]-8-methylaminoguanine	>400	>400	133
(124)	9-[(2-hydroxyethoxy)methyl]-8-dimethylaminoguanine	>400	>400	13
(125)	9-[(2-hydroxyethoxy)methyl]-8-piperdylguanine	250	333	3.5
(126)	8-hydroxy-9-[(2-hydroxyethoxy)methyl]guanine	48	>100[c]	3.2
(130)	9-[(2-hydroxyethoxy)methyl]-8-methylguanine	>400	>400	>800
(106)	9-[(2-hydroxyethoxy)methyl]-N^6,N^6-dimethyladenine	400	350	> 0.9
(23)	9-[(2-hydroxyethoxy)methyl]adenine	49	22	2.2
(111)	9-[(2-hydroxyethoxy)methyl]hypoxanthine	>100	>100[c]	-

.....continued

TABLE V (continued)

(108)	2-amino-6-chloro-9-[(2-hydroxyethoxy)methyl]purine	50	>100[c]	25
(102)	9-[(2-acetoxyethoxy)methyl]-6-chloropurine	60	62	3
(104)	9-[(2-acetoxyethoxy)methyl]-2,6-dichloropurine	10	2	1
(103)	9-[(2-acetoxyethoxy)methyl]-2-amino-6-chloropurine	55	71[c]	2.7
Reference compounds				
Acyclovir	9-[(2-hydroxyethoxy)methyl]guanine	12	13(21)[b]	300
IDU	5-iodo-2'-deoxyuridine	2	0.3	3
BVDU	(E)-5-(2-Bromovinyl)-2'-deoxyuridine	73	27	3857

[a]Inhibitory concentration$_{50}$ = concentration required to reduce cell growth, or (^{3}H-methyl)deoxythymidine (dThd) or (^{3}H-1',2')deoxyuridine (dUrd) incorporation by 50%. [b]As reported previously.[15] [c](^{14}C-2)dUrd instead of (^{3}H-1',2')dUrd. [d]Ratio of IC$_{50}$ for dThd or dUrd incorporation (whichever was lowest) to IC$_{50}$ for HSV-1, HSV-2 or VV (whichever was lowest; see Tables II and III).

3. Acid Hydrolyses

All hydrolyses were effected in 1 M HCl at 77°C with the exceptions of the pH/rate and temperature/rate studies on (22) and (131). This allowed direct comparison of rates but led to very long half lives for the more stable molecules. Kinetic data were obtained by direct UV absorbance measurements of the hydrolysis solutions (method A), UV absorbance of neutralised aliquots (method C) and by HPLC analysis of neutralised aliquots (method B). In all three methods the curves obtained were analysed by a non-linear first order computer fit, which has the advantage of not requiring an "infinite time" value. First order behaviour was checked by plotting "ln (a-x)" against "t" as given in equation (3), where "a" is the amount of starting material at time "t=0" and "x" is the amount of product at any time "t". In all cases linearity was observed.

$$A \xrightarrow{k} P \qquad (1)$$

$$\frac{dx}{dt} = k(a-x) \qquad (2)$$

$$\ln (a-x) = -kt + \ln a \qquad (3)$$

The half life of a reaction ($t_{\frac{1}{2}}$) is often quoted, and represents the time at which half of the starting

material has been consumed by the reaction, such that equation (3) can be reduced to equation (4).

$$\frac{\ln 2}{k} = t_{\frac{1}{2}} \qquad (4)$$

The hydrolysis of nucleosides follows a more complicated mechanism (eg. Scheme II), but it can be shown (Appendix) that the expected behaviour should still be pseudo first order at constant pH such that:

$$\text{Rate} = k_{obs}\,[S] \qquad (5)$$

where

$$k_{obs} = \frac{Ka_1[H^+]\left(k_1 + \frac{k_2[H^+]}{Ka_1}\right)}{Ka_1Ka_2 + [H^+]Ka_1 + [H^+]^2}$$

A kinetically indistinguishable result is obtained if the Schiff base mechanism (Scheme I) is assumed.

It was found that the (2-hydroxyethoxy)methyl-nucleosides were more stable to hydrolysis than either their 2'-deoxy or ribonucleoside counterparts (Tables VI and VII). This can be rationalised by either of the two proposed mechanisms (Scheme I or Scheme II) since both contain intermediates [(135) and (136)] requiring the stabilisation of a primary instead of a secondary

TABLE VI. Rate Constants of hydrolyses of (2-hydroxyethoxy)methyl purines and imidazoles in 1 M HCl at 77°C.

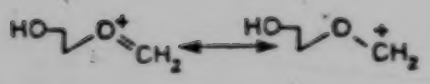
Compound	Rate Constant($mins^{-1}$)	Half Life(mins)	Relative Rate
(120) 8-chloro-9-[(2-hydroxyethoxy)methyl]guanine	$(5.0 \pm .3) \times 10^{-1}$	1.39±.08	11
(119) 8-bromo-9-[(2-hydroxyethoxy)methyl]guanine	$(3.98 \pm .09) \times 10^{-1}$	1.74±.04	9
(125) 9-[(2-hydroxyethoxy)methyl]-8-piperdylguanine	$(2.97 \pm .08) \times 10^{-1}$	2.33±.06	6.8
(124) 9-[(2-hydroxyethoxy)methyl]-8-dimethylaminoguanine	$(2.83 \pm .02) \times 10^{-1}$	2.45±.02	6.4
(129) 8-tert-butyl-9-[(2-hydroxyethoxy)methyl]guanine	$(2.08 \pm .03) \times 10^{-1}$	3.33±.05	5
(121) 9-[(2-hydroxyethoxy)methyl]-8-iodoguanine	$(1.75 \pm .01) \times 10^{-1}$	3.96±.01	4
(131) 9-[(2-hydroxyethoxy)methyl]-7-methylguanine	$(6.9 \pm .3) \times 10^{-2}$	10.0 ±.4	1.6
(106) 9-[(2-hydroxyethoxy)methyl]-N^6,N^6-dimethyladenine	$(4.8 \pm .2) \times 10^{-2}$	14.4 ±.6	1.1
(22) 9-[(2-hydroxyethoxy)methyl]guanine	$(4.4 \pm .1) \times 10^{-2}$	15.8 ±.4	1
(23) 9-[(2-hydroxyethoxy)methyl]adenine	$(3.2 \pm .3) \times 10^{-2}$	22. ± 2	0.73
(111) 9-[(2-hydroxyethoxy)methyl]hypoxanthine	$(1.6 \pm .2) \times 10^{-2}$	43 ± 5	0.36
(130) 9-[(2-hydroxyethoxy)methyl]-8-methylguanine	$(1.26 \pm .02) \times 10^{-2}$	55 ± 1	0.3
(122) 8-amino-9-[(2-hydroxyethoxy)methyl]guanine	$(5.1 \pm .1) \times 10^{-3}$	136 ± 3	0.12
(123) 9-[(2-hydroxyethoxy)methyl]-8-methylaminoguanine	$(3.9 \pm .1) \times 10^{-3}$	178 ± 5	0.09
(126) 8-hydroxy-9-[(2-hydroxyethoxy)methyl]guanine	$(8 \pm 1) \times 10^{-5}$	8,600 ± 1,000	0.002
(115) 4-carbamoyl-1-[(2-hydroxyethoxy)methyl]-5-methylaminoimidazole	$(4.57 \pm .08) \times 10^{-2}$	15.2 ±.3	1.04
(118) 5-amino-4-carbamoyl-1-[(2-hydroxyethoxy)-methyl]imidazole	$(1.77 \pm .02) \times 10^{-3}$	392 ± 4	0.04

TABLE VII. Rate Constants of hydrolyses of (2-hydroxyethoxy)methyl pyrimidines in 1 M HCl at 77°C.

Compound	Rate Constant($mins^{-1}$)	Half Life(mins)	Relative Rate
(96) 1-[(2-hydroxyethoxy)methyl]pyrimidin-4-one	$(7.28\pm0.3) \times 10^{-2}$	9.52±.04	214
(90) 1-[(2-hydroxyethoxy)methyl]isocytosine	$(1.82\pm.04) \times 10^{-3}$	390±8	5.4
(100) 3-[(2-hydroxyethoxy)methyl]cytosine	$(8.39\pm.09) \times 10^{-4}$	826±9	2.5
(80) 1-[(2-hydroxyethoxy)methyl]uracil	$(3.4 \pm.1) \times 10^{-4}$	2,040±60	1
(81) 1-[(2-hydroxyethoxy)methyl]thymine	$(2.1 \pm.06) \times 10^{-4}$	3,300±90	0.6
(86) 1-[(2-hydroxyethoxy)methyl]-5-nitrouracil	$(3.3 \pm.3) \times 10^{-5}$	21,000±2,000	0.1
(82) 5-fluoro-1-[(2-hydroxyethoxy)methyl]uracil	$(2.3 \pm.1) \times 10^{-5}$	30,000±1,300	0.07
(84) 5-bromo-1-[(2-hydroxyethoxy)methyl]uracil	$(1.9 \pm.1) \times 10^{-5}$	37,000±2,000	0.06
(83) 5-chloro-1-[(2-hydroxyethoxy)methyl]uracil	$(9.4 \pm.5) \times 10^{-6}$	74,000±4,000	0.03
(89) 1-[(2-hydroxyethoxy)methyl]-5-nitrocytosine	$(8.7 \pm.1) \times 10^{-3}$[b]	80±1	26
(94 5-bromo-1-[(2-hydroxyethoxy)methyl]cytosine	$(1.06\pm.06) \times 10^{-3}$[c]	650±40	3.1
(93) 5-chloro-1-[(2-hydroxyethoxy)methyl]cytosine	$(9.6 \pm.02) \times 10^{-4}$[c]	722±2	2.8
(88) 1-[(2-hydroxyethoxy)methyl]cytosine	1.4×10^{-5}[a,b]	49,400	0.04

[a]Value represents an upper limit (see text). [b]Both hydrolysis and deamination are occurring. The rate constant represents disappearance of starting material. [c]Only deamination was observed. The rate constant represents disappearance of starting material.

heteroatom-linked carbonium ion. Psicofuranine (137), which would hydrolyse via a tertiary oxocarbonium ion



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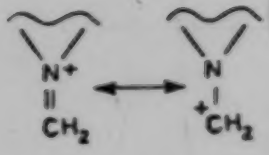
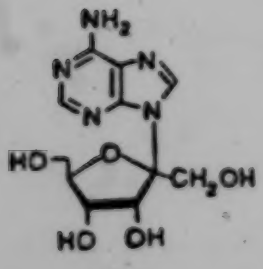


(136)

(138), has been found to be much more labile in aqueous acid than adenosine,[211,212] a fact consistant with this hypothesis. This effect may be reflected in the



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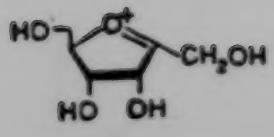


(138)

enthalpy of activation ($\Delta H^{\ddagger}$) of the reaction, as given by equation (7). The enthalpy value and the entropy of activation ($\Delta S^{\ddagger}$) are readily obtainable from a plot of

$$k = \frac{kT}{h} e^{-\frac{\Delta H^{\ddagger}}{RT}} . e^{\frac{\Delta S^{\ddagger}}{R}} \qquad (7)$$

"ln k" against the reciprocal of absolute temperature (T). The thermodynamic measurements for (22) (Table VIII) gave values of $\Delta H^{\ddagger}$ = 25 Kcal/mol and $\Delta S^{\ddagger}$ = 7.3 cal/mol deg. The enthalphy of activation found for (22) is higher than reported values for 2'-deoxy- and guanosine.[230] This is consistent with the lower expected stability of (135) relative to furanose oxocarbonium ion species. The positive entropy of activation indicates an increase in disorder in attaining the transition state. This is in harmony with both proposed mechanisms (Schemes I and II) and suggests significant C-N (or C-O) bond cleavage before participation of the solvent.

A linear dependence of the rate of hydrolysis of (22) with pH was observed in the range of pH 0 to 2.75, which includes the pKa[335] (Figure 2, Table IX). This rather limited range was not extended to higher pH values due to the slow rate of reaction. Similar results have

TABLE VIII. Variation of Rate Constant of Hydrolysis of (22) with Temperature.

Temperature (K)	Rate Constant ($mins^{-1}$)	Half Life (mins)
350	$(4.4 \pm .1) \times 10^{-2}$	15.8±.4
343	$(2.32 \pm .02) \times 10^{-2}$	29.9±.3
338	$(1.30 \pm .01) \times 10^{-2}$	53 ±.4
333	$(6.7 \pm .1) \times 10^{-3}$	103 ± 2
328	$(3.76 \pm .03) \times 10^{-3}$	184 ± 1
323	$(2.00 \pm .01) \times 10^{-3}$	347 ± 2

TABLE IX. Variation of Rate Constant of Hydrolysis of (22) with pH.

pH	Rate Constant ($mins^{-1}$)	Half Life (mins)
0	$(4.4 \pm .1) \times 10^{-2}$	15.8±.4
0.5	$(1.21 \pm .01) \times 10^{-2}$	57.3±.5
1.1	$(4.6 \pm .1) \times 10^{-3}$	151 ± 3
1.5	$(1.63 \pm .07) \times 10^{-3}$	425 ± 3
2.0	$(4.18 \pm .06) \times 10^{-4}$	1660 ± 24
2.35	$(3.1 \pm .1) \times 10^{-4}$	2240 ± 70
2.75	$(4.2 \pm .4) \times 10^{-5}$	16500 ± 1600

TABLE X. Variation of Rate Constant of Hydrolysis of (22) with Formate Buffer Strength at pH 2.75.

Buffer Strength (molar)	Rate Constant ($mins^{-1}$)	Half Life (mins)
0.15	$(3.4 \pm .3) \times 10^{-5}$	20400±1,800
0.3	$(4.2 \pm .4) \times 10^{-5}$	16500±1,600
0.6	$(3.7 \pm .4) \times 10^{-5}$	18700±2,000

TABLE XI. Variation of Rate Constant of Hydrolysis of (131) with pH.

pH	Rate Constants ($mins^{-1}$)	Half Life (mins)
0	$(6.9 \pm .3) \times 10^{-2}$	10.0±.4
0.7	$(1.80 \pm .02) \times 10^{-2}$	38.5±.4
1.1	$(8.0 \pm 1) \times 10^{-3}$	87 ± 10
1.65	$(2.71 \pm .01) \times 10^{-3}$	256 ± 9
2.00	$(1.61 \pm .01) \times 10^{-3}$	431 ± 3
2.35	$(8.7 \pm .2) \times 10^{-4}$	797 ± 18
3.00	$(4.92 \pm .06) \times 10^{-4}$	1410 ± 17
3.50	$(4.6 \pm .1) \times 10^{-4}$	1510 ± 33
4.00	$(4.15 \pm .03) \times 10^{-4}$	1670 ± 12
4.50	$(3.8 \pm .2) \times 10^{-4}$	1800 ± 100
5.00	$(4.17 \pm .02) \times 10^{-4}$	1660 ± 8
5.50	$(4.00 \pm .02) \times 10^{-4}$	1730 ± 9
6.00	$(3.77 \pm .02) \times 10^{-4}$	1840 ± 10
6.40	$(3.8 \pm .2) \times 10^{-4}$	1800 ± 100
6.80	$(5.4 \pm .1) \times 10^{-4}$	1280 ± 24
7.10	$(1.08 \pm .07) \times 10^{-3}$	640 ± 40
7.60	$(3.36 \pm .03) \times 10^{-3}$	210 ± 2

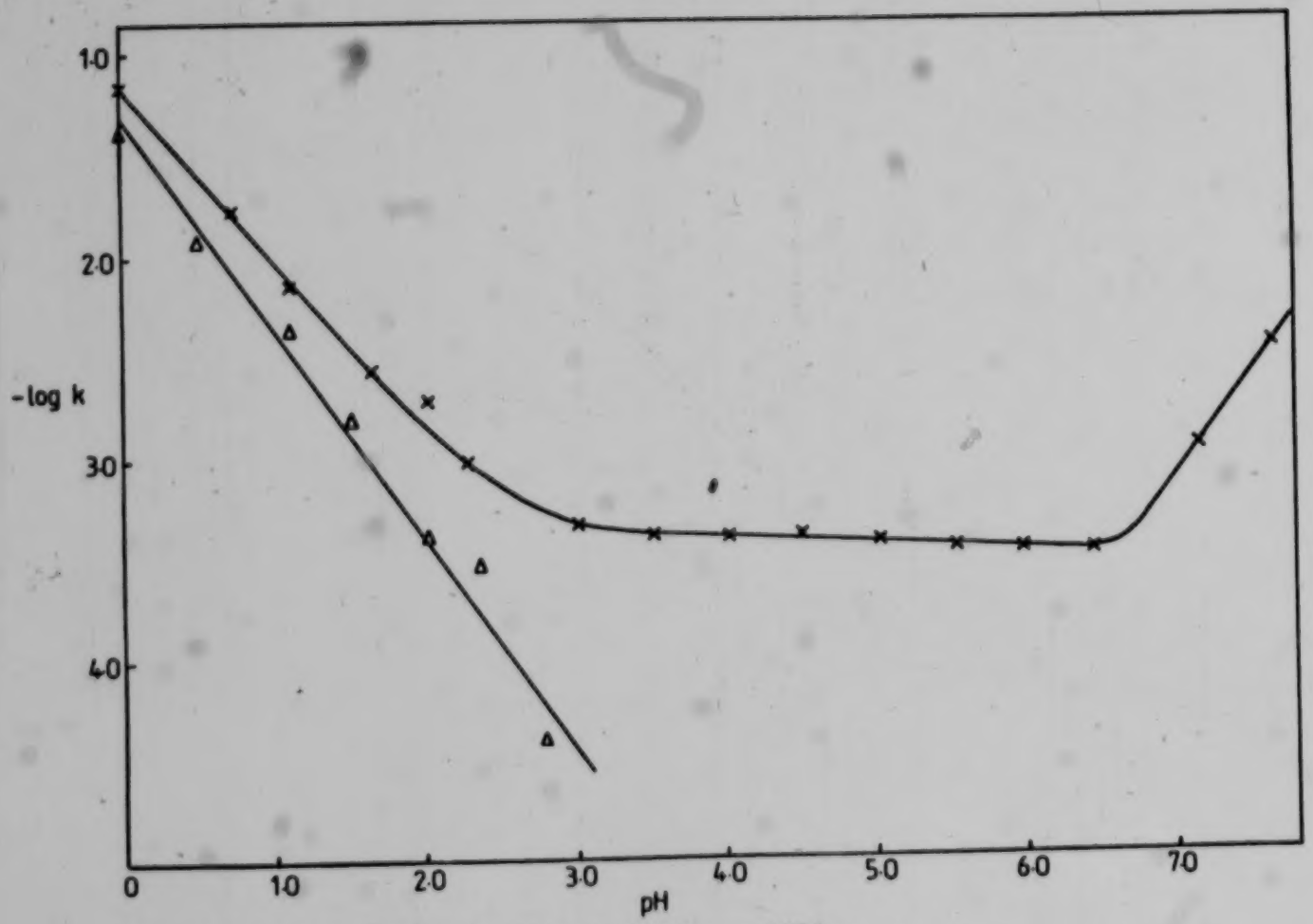
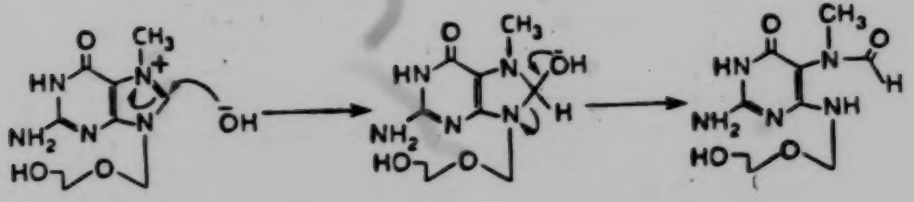


Figure 2: pH-rate profiles for the hydrolysis of (22) ▲ and (131) ×

been reported for the hydrolysis of 2'-deoxyguanosine and guanosine.[228-230] The pH-rate profile of 9-[(2-hydroxyethoxy)methyl]-7-methylguanine (131) was found to have three distinct regions (Figure 6, Table X); a) pH < 3, a pH dependent region in which a second protonation of the betaine (131) occurs; b) pH 3-6.5, a pH independent region in which spontaneous hydrolysis of (139) occurs, and c) pH > 6.5, ring opening of the imidazole moiety occurs (Scheme XIII). Analogous results have been observed previously with 7-methylguanosine.[228,336,337]

Scheme XIII



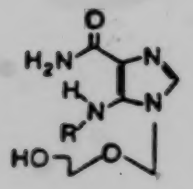
(139)

Compound (22) was hydrolysed in formate buffers (pH 2.75) of different strengths without any significant effect on the rate of hydrolysis (Table X). This was expected since only specific acid

catalysis has been observed in the hydrolysis of nucleosides.

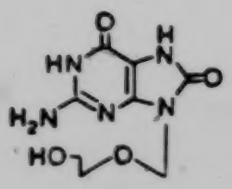
The effect of various substituents at the 8-position of (22) on the rate of hydrolysis was examined (Table VI). Without determinaton of the pH profile or pKa data for each compound, it is not possible to estimate the relative amounts of mono and diprotonation. With this limitation in mind, these results can be explained by two factors: (1) the basicity of the departing N-9 atom and (2) steric strain. The presence of electron withdrawing groups such as iodine (121), bromine (119) and chlorine (120) caused rate enhancements in accord with their respective electronegativities. Electron donating groups at the 8-position such as methyl (130), amino (122) and methylamino (123) caused rate decreases. However, as the size of the substituent increased, steric strain became an important factor. The dimethylamino (124) and piperdyl (125) substituents caused considerable rate enhancements, a result which would not have been expected on electronic grounds (diprotonation of the imidazole amidine system is unlikely under the hydrolysis conditions employed). A similar effect also was noted with the tert-butyl group (129). The methylamino group (123) may be considered to be a medium

sized substituent, but it can rotate to adopt a strain free conformation not available to (124), (125) and (129). The most unusual result in this series of substrates is the slow hydrolysis of the 8-hydroxy compound (126). Since the keto tautomeric form (126) is predominant, protonation at N-7 (now an amide-type nitrogen) would be difficult and would more likely occur at N-3. Thus, in the hydrolysis of (126) the developing negative charge at N-9 cannot be resonance stabilised by a positively charged amidine system as with the usual 7-protonated purines. The leaving group would be more basic and retard the rate of hydrolysis.



(118) R= H

(115) R= CH_3



(126)

The formation of deprotonated species would be difficult since only amide-type ring nitrogens and normally non-protonated exocyclic heteroatoms are present.

Of the two 1-[(2-hydroxyethoxy)methyl]imidazoles studied, (115) hydrolysed faster than (118). This is difficult to explain by electronic factors. The steric crowding caused by the methyl group may be the reason for the increase in rate for (115).

In contrast to the report of Garrett on pyrimidine nucleoside hydrolyses,[219] the introduction of a substituent at the 5-position of 1-[(2-hydroxyethoxy)-methyl]uracil (80) caused a decrease in the rate of hydrolysis (Table VII). As discussed in the introduction of this thesis, it is likely that uracil nucleosides can protonate at either the base or sugar moiety, thus making both pathways (Schemes I and II) available. A change of substituent at C5 may cause a shift in mechanism in this series. The rates of the 1-[(2-hydroxyethoxy)methyl]-5-substituted uracils can be rationalized by such a postulate. A substituent that reduces the electron density in the pyrimidine ring would favour its dissociation via the oxocarbonium ion pathway (Scheme II). This is consistent with a decreasing trend when comparing the rate of the 5-nitro (86), 5-fluoro (82) and 5-chloro (83) compounds. If the only mechanism possible were this oxocarbonium ion pathway, then the trend should extend to the bromo (84) methyl (81) and unsubstituted (80) compounds. However,

as the electron density of the pyrimidine ring increases, the Schiff base pathway must become significant. The bromo derivative (84) would represent the start of this trend, and probably hydrolyses via both pathways. Whether (80) and (81) hydrolyse exclusively by the Schiff base pathway (Scheme I) was not determined. The hydrolysis rate of 1-[(2-hydroxyethoxy)-methyl]-5-iodouracil (85) was of key interest, but deiodination occurred under the hydrolysis conditions.

To investigate further the Schiff base possibility, the solvolysis of (80), (81), (86) and (22) were effected in anhydrous methanolic hydrogen chloride. If a Schiff base intermediate were formed, it might be trapped under these conditions.[260,263] In the case of (80) and (81) trapping was observed and the uracil and thymine derivatives (73) and (74) were detected.* No such products ((75) and (140)) were observed in solvolyses of (86) and (22) under identical conditions. It may be argued that changing the solvent may also alter the reaction pathway. However, since specific acid catalysis was observed and little participation of solvent before the transition state occurred, this

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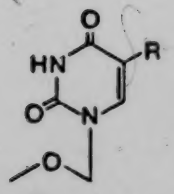
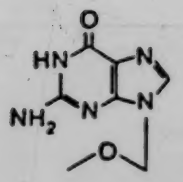
* By HPLC comigration with synthetic samples.



(73) R = H
(74) R = CH_3
(75) R = NO_2



(140)

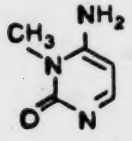
seems unlikely. Compound (80) hydrolyses faster than (81), which would not be expected if a Schiff base pathway were operating exclusively. One possible explanation is that the electron donating properties of the methyl group facilitate protonation of the pyrimidine ring; an event that would retard Schiff base formation. Comparison of the rates of hydrolysis of the 1-[(2-hydroxyethoxy)methyl]-5-substituted uracils with their 2'-deoxyribosyl counterparts[219] shows that the retardations are not of the same order of magnitude. For example, the 1-[(2-hydroxyethoxy)methyl]uracil compounds with an electron withdrawing substituent at the 5-position show a > 100-fold retardation in rate relative to the corresponding 2'-deoxynucleosides[219] whereas 1-[(2-hydroxyethoxy)methyl]uracil (80)

hydrolyses at one half the rate of 2'-deoxy-uridine. This is consistent with the operation of different mechanisms. The Schiff base intermediate (136) may be destabilised to a lesser extent than the oxocarbonium ion intermediate (135), due to the greater ability of nitrogen to stabilise an adjacent positive charge.[338]

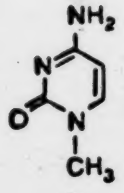
Cytidine is the most acid labile of the natural pyrimidine nucleosides, and likely hydrolyses via the oxocarbonium ion pathway.[218] Our results support this. Although the hydrolysis of 1-[(2-hydroxyethoxy)-methyl]cytosine (88) was complicated by deamination of the base to give uracil by-products, the rate of disappearance of starting material was taken as an "upper limit", i.e. the hydrolysis rate could be no faster than that of the overall disappearance of (88). This rate is at least 100-fold slower than that of 2'-deoxycytidine (12) and 25-times slower than that of (80). The introduction of electron withdrawing groups at the 5-position of the cytosine ring would be expected to cause rate enhancements. Unfortunately, these groups also increased the rate of deamination. Treatment of the 5-chloro (93) and 5-bromo (94) derivatives resulted in complete deamination before any hydrolysis of the side-chain was detected. Glycosyl

cleavage did compete with deamination in the hydrolysis of 5-nitro-1-[(2-hydroxyethoxy)methyl]cytosine (89) indicating approximately a two orders of magnitude rate enhancement over that of the unsubstituted compound (88).

Deamination was not a problem with the hydrolysis of 3-[(2-hydroxyethoxy)methyl]cytosine (100). The marked increase in rate of hydrolysis of (100) as compared to the N-1 isomer (88) is readily explained by the basicities of the leaving groups. Suitable models are N-3-methylcytosine (pKa 7.38)[269] (45) and N-1-methylcytosine (pKa 4.55) (141).[269]

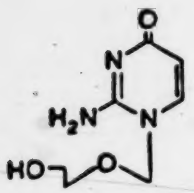


(45)

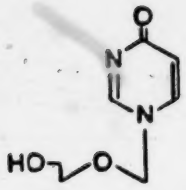


(141)

The importance of the basicity of the leaving group also was reflected in the rates of hydrolysis of (90) and (96). Both are readily protonated at N3 of the heterocycle but the 2-amino substituent in (90) causes a significant increase in basicity at the departing N-1 position (N-3-methyl isocytosine



(90)



(96)

pK 4.2,[269] N-3-methyl pyrimid-4-one pKa 1.84[266]). Thus (96) was found to hydrolyse 40-times faster than (90). This fact, in addition to being consistent with the oxocarbonium ion pathway (Scheme II), contradicts Dekker's suggestion that intramolecular proton transfer is an important consideration in the mechanism of hydrolysis of isocytidine.[208]

Summary of Kinetic Results

The acid catalysed hydrolysis of purine nucleosides is now generally accepted to occur via an oxocarbonium ion intermediate (Scheme II).[224,225,228-231,233] The relative rates of hydrolysis of the present series of 9-[(2-hydroxyethoxy)methyl]purines were consistent with such a mechanism, as were the pH/rate profile and temperature study with (22) and (131). Replacement of the ribose moiety with the (2-hydroxyethoxy)methyl

group did not appear to change the mechanism of hydrolysis, but merely retarded the rates of reaction.

The markedly slower rate of hydrolysis in the (2-hydroxyethoxy)methyl cytosine compounds allowed deamination to compete. However, it was clear that electron withdrawing substituents increased the rate of hydrolysis in harmony with the "oxocarbonium ion" mechanism. This is in agreement with work of Shapiro and Danzig[218] and Lonneberg[221] on hydrolyses of cytidine nucleosides. The comparatively fast rates of hydrolysis of 3-[(2-hydroxyethoxy)methyl]cytosine (100) and 1-[(2-hydroxyethoxy)methyl]cytosine (90) are in harmony with the more facile departure of less basic heterocyclic leaving groups. This has clarified the situation with respect to the hydrolysis of nucleosides (pyrimidines as well as purines) with readily protonated bases (pka >1).

In contrast to these consistent results with purine and cytosine nucleosides, little is known about the detailed hydrolysis mechanism for uracil nucleosides. Our trapping experiments and comparisons of rates of hydrolysis of 5-substituted-1-[(2-hydroxyethoxy)methyl]uracils suggest the operation of at least two mechanisms (Schemes I and II). The nature of the base and/or sugar substituents appears to control which

pathway predominates. Such an explanation not only allows an interpretation of our results (but also provides a plausible rationalisation of previously published work (see introduction).[217-219,260-264]

Scheme III summarises several pathways available to nucleosides in aqueous acid solutions. For predictions regarding the acid stability of a particular nucleoside, factors such as the possible site(s) of protonation and the basicity of the leaving group appear to be more significant than whether the base is a pyrimidine or a purine. Further studies on pyrimidine nucleoside analogues having bases with a range of pka values for both protonation (pka_1) and departure (pka_2) and graded stabilities of the oxocarbonium ion fragment might allow clarification of further mechanistic details. Cytosine analogues with decreased pka_2 values but stable towards deamination would be useful for further corroboration of the mode of hydrolysis of this type of nucleoside.

EXPERIMENTAL

a) GENERAL PROCEDURES

Melting points were determined on a Reichert microstage apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on Varian HA-100, Bruker WH-200 or Bruker WH-400 spectrometers operating in the FT mode, with tetramethylsilane as internal reference normally in deuterated dimethylsulphoxide (DMSO) unless specified otherwise. Ultraviolet (UV) spectra were recorded on a Cary 15 spectrophotometer. Mass spectra (MS) were determined by the mass spectrometry laboratory of this department on an AEI MS-50 instrument with computer processing at 70 eV using a direct probe for sample introduction. Elemental analyses were determined by the microanalytical laboratory of this department. Evaporations were effected using Buchler and Büchi rotating evaporators equipped with Dewar "dry ice" condensers under water or mechanical oil pump vacuum, at 40°C or cooler. Thin layer chromatography (TLC) was performed on E. Merck chromatographic sheets (silica gel 60 F_{254}, layer thickness 0.2 mm, catalogue No. 5775) with sample observation under UV light (2537 Å). Preparative layer chromatography (PLC) was performed on glass plates

coated with Merck silica gel PF 254. The solvents used for TLC were: different ratios of methanol and chloroform (1:50, 1:20, 1:10), SSE (the upper phase of EtOAc:n-PrOH:H_2O, 4:1:2) and i-PrOH:H_2O:NH_4OH (7:2:1). Column chromatography was performed using Mallinckrodt CC-7 (200 mesh) silica gel and Barnebey-Cheney Au-4 charcoal. The charcoal was conditioned by washing with methanol then chloroform and allowing it to dry. This was followed by refluxing it in aqueous HCl (1 molar), with the acid solution being replaced periodically until the supernatant solution remained colourless, followed by similar sequential refluxing in 10% aqueous sodium hydroxide. Finally the charcoal was washed with water until neutral, methanol, chloroform and air dried. All solvents used were reagent grade and were distilled prior to use. Purification of most solvents and reagents was accomplished as described in references 339 and 340. Dried solvents were stored over Davison 3 and 4 Å molecular sieves purchased from the Fisher Scientific Company.

The syntheses of (2-hydroxyethoxy)methyl-nucleosides from appropriate heterocycles generally involved two steps, the coupling reaction and the subsequent deacylation. Three procedures were developed for each transformation and are described in

detail for compounds (64), (103), (110), (80), (82), (96). These are designated as Coupling methods A, B and C and Deprotection methods A, B and C, respectively. Subsequent experimental descriptions will refer to these methods.

Buffer solutions were formulated to 1.0 M from commercially available materials: pH 0-2.3, HCl; pH 3-4.5, formic acid; pH 4.5-5.5, acetic acid; pH 6.0-7.6, monosodium phosphate. Ionic strength was maintained at 1.0 M by the addition of sodium chloride. pH readings were made with a Radiometer GK 2321C combination electrode standardised with fischer certified buffers (pH 4.00, 7.00 and 10.00) immediately before use. pH values were measured at room temperature and no corrections were made for their use at elevated temperatures. Three methods were employed for the collection of the kinetic data:

Method A; faster kinetic data ($k > 3 \times 10^{-4}$ $mins^{-1}$) were obtained using a Varian/Cary 210 UV/visible spectrophotometer interfaced to an Apple II microcomputer. This arrangement allowed collection of absorbance/time data for up to 5 cells and analysed the kinetic curves as being first order with a standard non-linear leasts squares method. Reactions were

initiated by injecting 100 µL of a nucleoside stock solution into 3 mL of the appropriate hydrolysis solution that was maintained at 77 ± 0.2°C, in a 1 cm length quartz cuvette. The pH values of the initial and final hydrolysis solutions were measured and found to be invariant.

Method B; slower kinetic data ($k < 3 \times 10^{-4}$ $mins^{-1}$), or data from hydrolysis solutions which had only small changes in the UV spectrum, were obtained by the following procedure. Appropriate hydrolysis solutions (50 µL) were sealed in capillary tubes and immersed in a Colora constant temperature bath at 77 ± 0.5°C. The reaction was halted by breaking a tube in 0.05 N sodium hydroxide solution (1 mL). A 100 µL portion of this "quenched solution" was then analysed on a Waters high performance liquid chromatograph (HPLC) fitted with a Columbia Scientific Industries digital mini-Lab integrator model CSI 38. Separation of products was achieved on a reversed phase column (Whatman PxS-1025 ODS-2, 4.6 mm x 25 cm) with a mobile phase of 0.1 m $NH_4H_2PO_4$ in various mixtures of acetonitrile and water (1:40, 1:20, 1:10) at a flow rate of 3 mL/min (3,000 p.s.i.). The system was calibrated at a particular wavelength for each nucleoside studied. A

molar ratio of starting material to final product was determined in order to eliminate errors due to dilution. Analysis of the kinetic curves was effected using a standard non-linear least squares method.

Method C; sodium hydroxide "quenched solutions" were obtained as described in "Method B". The analysis of these solutions was performed by measuring the progressive change in UV absorbance at a specified wavelength. The absorbance/time data was analysed with a standard non-linear least squares method.

b) SYNTHESIS

(2-Acetoxyethoxy)methyl bromide (61)

Freshly distilled acetyl bromide (13 g, 106 mmol) was stirred with cooling in an ice bath while 7.4 g (100 mmol) of 1,3-dioxolane (59) was added slowly. A rapid exothermic reaction occurred giving quantitative conversion to (61) (as judged by ^{1}H NMR). Vacuum distillation of this material gave 17.4 g (88%) of (61); bp 58-60°C/0.1 Torr; ^{1}H NMR ($CDCl_3$, 100 MHz) δ 2.10 (s, 3, CH_3CO), 3.88 and 4.27 (A_2B_2 multiplet, 2 and 2, OCH_2CH_2OAc), 5.72 (s, 2, OCH_2Br); ^{13}C NMR ($CDCl_3$, 22.6 MHz) δ 20.80 (CH_3CO), 62.14 and 69.22

(AcOCH$_2$CH$_2$), 75.60 (OCH$_2$Br), 170.69 (CH$_3$CO). MS (CI) m/z 214, 216 (M^+ + 18[^{79}Br,^{81}Br]). Anal. Calcd. for $C_5H_9BrO_3$: C, 30.48; H, 4.60; Br, 40.55. Found: C, 30.55; H, 4.59; Br, 40.72.

Coupling Method A

1-[(2-Acetoxyethoxy)methyl]uracil (64)

To a suspension of 168 mg (1.5 mmol) of uracil (5) in 5 mL of hexamethyldisilazane was added a drop of chlorotrimethylsilane and the stirred mixture was heated at reflux with exclusion of moisture until a clear solution was obtained. Excess silylating agent was removed in vacuo with protection against moisture. The residual clear oil was dissolved in 15 mL of dry acetonitrile and cooled to 0°C. A solution of 197 mg (1 mmol) of (2-acetoxyethoxy)methyl bromide (61) in 5 mL of dry acetonitrile was added slowly with stirring. The solution was allowed to stir for 2 h while warming to room temperature, at which time TLC (silica gel, 10% MeOH/$CHCl_3$) indicated complete reaction. Volatile materials were evaporated in vacuo. The resulting yellow oil was chromatographed on a column (20 g silica gel, 3 cm diameter) using 2% MeOH/$CHCl_3$ as eluant. Fractions containing (64) were

combined and evaporated. The residue was crystallised from $CHCl_3/Et_2O$ to give 181 mg (79%) of (64); mp 77-78°C; ^1H NMR ($CDCl_3$, 100 MHz) δ 2.07 (s, 3, CH_3), 3.81 and 4.23 (A_2B_2 multiplet*, 2 and 2, OCH_2CH_2OAc), 5.21 (s, 2, NCH_2O), 5.80 (d, J = 8 Hz, 1, H5), 7.33 (d, J = 8 Hz, 1, H6), 9.4-9.7 (broad s, 1, NH). MS m/z 228.0747; calcd. for M^+: 228.0742. UV (0.1 N HCl) max 259 nm (ε 9800), min 228 nm (ε 2300); (0.1 N NaOH) max 259 nm (ε 6800), min 243 nm (ε 5700). Anal. Calcd. for $C_9H_{12}N_2O_5$: C, 47.37; H, 5.30; N, 12.28. Found: C, 47.09; H, 5.39; N, 12.16.

Deprotection Method A

1-[(2-Hydroxyethoxy)methyl]uracil (80)

A 228 mg sample of 1-[(2-acetoxyethoxy)methyl]-uracil (64) was added to 40 mL of MeOH saturated with NH_3 at 10°C. The flask was stoppered tightly and the solution was stirred at room temperature for 5 h. TLC (10% MeOH/$CHCl_3$) indicated that complete deprotection of (64) had occurred. Volatile materials were evaporated and the residue was crystallised from MeOH to give 169 mg (90%) of (80); mp 147-148°C; ^1H NMR (DMSO-d^6, 100 MHz) δ 3.48 (s, 4, OCH_2CH_2O), 4.64 (broad s, 1,

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* Rigorously named an AA'BB' system.

7.67 (d, J = 8 Hz, 1, H6), 11.25 (broad s, 1, NH). MS m/z 186.0640; calcd. for M^+: 186.0641. UV (0.1 N HCl) max 259 nm (ε 9700), min 229 nm (ε 2500); (0.1 N NaOH) max 260 nm (ε 6700), min 243 nm (ε 5400). Anal. Calcd. for $C_7H_{10}N_2O_4$: C, 45.16; H, 5.41; N, 15.05. Found: C, 44.93; H, 5.33; N, 15.06.

1-[(2-Acetoxyethoxy)methyl]thymine (65)

Under the conditions outlined in "coupling method A" 189 mg (1.5 mmol) of thymine were coupled with 197 mg (1 mmol) (2-acetoxyethoxy)methyl bromide (61). The crude product was chromatographed on silica gel, with 2% MeOH/$CHCl_3$ as elutant, followed by crystallisation from $CHCl_3/Et_2O$ to give 203 mg (84%) of (65); mp 123-125°C; ^1H NMR (DMSO-d^6, 400 MHz) δ 1.78 (d, J = 1.2 Hz, 3, C5-CH_3), 2.01 (s, 3, CH_3CO), 3.70 and 4.11 (A_2B_2 multiplet, 2 and 2, OCH_2CH_2OAc), 5.08 (s, 2, NCH_2O), 7.56 (q, J = 1.2 Hz, 1, H6), 11.5 (broad s, 1, NH). MS m/z 242.0903; calcd. for M^+: 242.0943. UV (0.1 N HCl) max 264 nm (ε 9600) min 233 nm (ε 2500); (0.1 N NaOH) max 265 nm (ε 7000) min 246 nm (ε 4800). Anal. Calcd. for $C_{10}H_{14}N_2O_5$: C, 49.57; H, 5.83; N, 11.56. Found: C, 49.32, H, 5.72; N, 11.57.

1-[(2-Hydroxyethoxy)methyl]thymine (81)

A 4 g (16.5 mmol) sample of 1-[(2-acetoxyethoxy)-methyl]thymine (65) was deacylated as described in "deprotection method B". The crude product was recrystallised from MeOH/Et_2O to give 3.077 g (93%) of (81); mp 150.5-152°C. ^1H NMR (DMSO-d^6, 400 MHz), δ 1.78 (d, J = 1.2 Hz, 1, C5-CH_3), 3.50 (s, 4, OCH_2CH_2O), 4.65 (broad s, 1, OH), 5.06 (s, 2, NCH_2O), 7.55 (q, J = 1.2 Hz, 1, H6), 11.5 (broad s, 1, NH). MS m/z 200.0801; calcd. for M^+: 200.0797. UV (0.1 N HCl) max 265 nm (ε 9000) min 234 nm (ε 2200); (0.1 N NaOH) max 264 nm (ε 6500) min 245 nm (ε 4400). Anal. Calcd. for $C_8H_{12}N_2O_4$: C, 48.00; H, 6.04; N, 13.99. Found: C, 48.00; H, 5.95; N, 13.95.

1-[(2-Acetoxyethoxy)methyl]-5-fluorouracil (66)

Under the conditions outlined in "coupling method A" 195 mg (1.5 mmol) of 5-fluorouracil (14) were coupled with 197 mg (1 mmol) of (61). The crude product was chromatographed on a silica gel column with 2% MeOH/$CHCl_3$ as eluant, followed by crystallisation from $CHCl_3$/Et_2O giving 207 mg (84%) of (66); mp 148-149°C; ^1H NMR (DMSO-d^6, 100 MHz) δ 1.98 (s, 3, CH_3), 3.74 and 4.13 (A_2B_2 multiplet, 2 and 2, OCH_2CH_2OAc), 5.04 (s, 2, NCH_2O), 8.10 (d, J = 6.6 Hz, 1, H6), 11.9

(broad s, 1, NH). MS m/z 246.0652; calcd. for M^+: 246.0640. UV (0.1 N HCl) max 266 nm (ε 8600) min 232 (ε 2000) (0.1 N NaOH) max 265 nm (ε 6200) min 248 nm (ε 5000). Anal. Calcd. for $C_9H_{11}FN_2O_5$: C, 43.90; H, 4.50; N, 11.38. Found: C, 43.68; H, 4.63 N, 11.20.

Deprotection Method B

5-Fluoro-1-[(2-hydroxyethoxy)methyl]uracil (82)

To 60 ml of dry MeOH was added 0.23 g (10 mmol) of sodium metal. After hydrogen evolution was complete 1.476 g (6 mmol) of (66) was added. Stirring was continued for 2 h at room temperature. TLC (10% MeOH/$CHCl_3$) indicated that complete deprotection of (66) had occurred. Amberlite IR-120 (H^+) resin was added until the solution was neutral to moist pH paper. The mixture was filtered, the resin washed with MeOH and the combined filtrate evaporated. The colourless residual powder was recrystallised from MeOH/H_2O to give 1.175 g (96%) of (82); mp 154-156°C; ^1H NMR (DMSO-d^6, 100 MHz), δ 3.48 (s, 4, OCH_2CH_2O), 4.61 (broad s, 1, OH), 5.05 (s, 2, NCH_2O), 8.10 (d, J = 6.6 Hz, 1, H6), δ 11.7 (broad s, 1, NH). MS m/z 204.0541; calcd. for M^+: 204.0546. UV (0.1 N HCl) max 266 nm (ε 8200) min 232 nm (ε 1800); (0.1 N NaOH) max

265 nm (ε 6100) min 248 nm (ε 4700). Anal. Calcd. for $C_7H_9FN_2O_4$: C, 41.18; H, 4.44; N, 13.72. Found: C, 41.12; H, 4.56; N, 13.63.

1-[(2-Acetoxyethoxy)methyl]-5-chlorouracil (67)

Under the conditions outlined in "coupling method A" 219.8 mg (1.5 mmol) of 5-chlorouracil were coupled with 197 mg (1 mmol) of (2-acetoxyethoxy)methyl bromide (61). The crude product was chromatographed on silica gel, with 2% MeOH/$CHCl_3$ as elutant, followed by crystallisation from $CHCl_3/Et_2O$ to give 228 mg (87%) of (67); mp 119-120°C; ^1H NMR (DMSO-d^6, 100 MHz) δ 2.01 (s, 3, CH_3), 3.73 and 4.11 (A_2B_2 multiplet, 2 and 2, OCH_2CH_2OAc), 5.11 (s, 2, NCH_2O), 8.20 (s, 1, H6), 11.9 (broad s, 1, NH). MS m/z 262.0359, 264.0337 (^{35}Cl, ^{37}Cl); calcd. for M^+: 262.0379, 264.0296 (^{35}Cl, ^{37}Cl). UV (0.1 N HCl) max 274 nm (ε 8700) min 238 nm (ε 1500); (0.1 N NaOH) max 271 nm (ε 5900) min 249 nm (ε 3600). Anal. Calcd. for $C_9H_{11}ClN_2O_5$: C, 41.16; H, 4.22; N, 10.67. Found: C, 41.10; H, 4.06; N, 10.66.

5-Chloro-1-[(2-hydroxyethoxy)methyl]uracil (83)

A 200 mg (0.76 mmol) sample of 1-[(2-acetoxyethoxy)methyl]-5-chlorouracil (67) was deacylated as described in "deprotection method B". The crude

product was recrystallised from 95% EtOH to give 164 mg (98%) of (83); mp 167-168°C; ^{1}H NMR (DMSO-d^6, 100 MHz), δ 3.50 (s, 4, OCH_2CH_2O), 4.30 (broad s, 1, OH), 5.08 (s, 2, NCH_2O), 8.16 (s, 1, H6). MS m/z 220.0248, 222.0227 (^{35}Cl,^{37}Cl). Calcd. for M^+: 220.0251, 222.0221. UV (0.1 N HCl) max 274 nm (ε 8500) min 238 nm (ε 1500) (0.1 N NaOH) max 272 nm (ε 5800) min 249 nm (ε 3500). Anal. Calcd. for $C_7H_9ClN_2O_4$: C, 38.11; H, 4.11; N, 12.70. Found: C, 37.88; H, 3.96; N, 12.42.

1-[(2-Acetoxyethoxy)methyl]-5-bromouracil (68)

Under the conditions outlined in "coupling method A" 286.5 mg (1.5 mmol) of 5-bromouracil were coupled with 197 mg (1 mmol) of (2-acetoxyethoxy)methyl bromide (61). The crude product was chromatographed on silica gel, with 2% MeOH/$CHCl_3$ as elutant, followed by crystallisation from $CHCl_3$/Et_2O to give 264 mg (86%) of (68); mp 131-132°C; ^{1}H NMR (DMSO-d^6, 100 MHz) δ 2.00 (s, 3, CH_3), 3.73 and 4.10 (A_2B_2 multiplet, 2 and 2, OCH_2CH_2OAc) 5.10 (s, 2, NCH_2O), 8.26 (s, 1, H6), 8.8 (broad s, 1, NH). MS (CI) m/z 324, 326 (M^+ + 18 [^{79}Br, ^{81}Br]). UV (0.1 N HCl) max 277 nm (ε 8600) min 241 nm (ε 1100); (0.1 N NaOH) max 274 nm (ε 5900) min 250 nm (ε 3300). Anal. Calcd. for $C_9H_{11}BrN_2O_5$: C, 35.20; H, 3.61; N, 9.12. Found: C, 35.04; H, 3.66; N, 8.99.

5-Bromo-1-[(2-hydroxyethoxy)methyl]uracil (84)

A 307 mg (1 mmol) sample of 1-[(2-acetoxyethoxy)-methyl]-5-bromouracil (68) was deacylated as described in "deprotection method A". The crude product was recrystallised from 95% EtOH to give 206 mg (78%) of (84); mp 152-153°C; ^{1}H NMR (DMSO-d^6, 100 MHz) δ 3.51 (s, 4, OCH_2CH_2O), 4.65 (broad s, 1, OH), 5.10 (s, 2, NCH_2O), 8.25 (s, 1, H6), 11.75 (broad s, 1, NH). MS m/z 263.9746, 265.9727 (^{79}Br, ^{81}Br). Calcd. for M^+: 263.9746, 265.9725. UV (0.1 N HCl) max 276 nm (ε 8600) min 241 nm (ε 1700); (0.1 N NaOH) max 274 nm (ε 5700) min 251 nm (ε 3300). Anal. Calcd. for $C_7H_9BrN_2O_4$: C, 31.72; H, 3.42; N, 10.57. Found: C, 31.91; H, 3.52; N, 10.46.

1-[(2-Acetoxyethoxy)methyl]-5-iodouracil (69)

Under the conditions outlined in "coupling method A" 357 mg (1.5 mmol) of 5-iodouracil were coupled with 197 mg (1 mmol) of (2-acetoxyethoxy)methyl bromide (61). The crude product was chromatographed on silica gel, with 2% MeOH/$CHCl_3$ as elutant, followed by crystallisation from $CHCl_3$/Et_2O to give 280 mg (79%) of (69); mp 121-123°C; ^{1}H NMR (DMSO-d^6, 100 MHz) δ 1.99 (s, 3, CH_3), 3.70 and 4.09 (A_2B_2 multiplet, 2 and 2, OCH_2CH_2OAc), 5.08 (s, 2, NCH_2O), 8.23 (s, 1, H6), 11.7

(broad s, 1, NH). MS m/z 353.9727. Calcd. for M^+: 353.9753. UV (0.1 N HCl) max 285 nm (ε 7000) min 246 nm (ε 2000); (0.1 N NaOH) max 276 nm (ε 5000) min 252 nm (ε 3300). Anal. Calcd. for $C_9H_{11}IN_2O_5$: C, 30.53; H, 3.13; N, 7.90. Found: C, 30.53; H, 3.18; N, 7.60.

1-[(2-Hydroxyethoxy)methyl]-5-iodouracil (85)

A 708 mg (2 mmol) sample of 1-[(2-acetoxyethoxy)-methyl]-5-iodouracil (69) was deacylated as described in "deprotection method B". The crude product was recrystallised from $CHCl_3/Et_2O$ to give 576 mg (92%) of (85); mp 175-176°C; ^1H NMR (DMSO-d^6, 100 MHz) δ 3.50 (s, 4, OCH_2CH_2O), 4.65 (broad s, 1, OH), 5.08 (s, 2, NCH_2O), 8.20 (s, 1, H6), 11.65 (broad s, 1, NH). MS m/z 311.9612. Calcd. for M^+: 311.9607. UV (0.1 N HCl) max 286 nm (ε 7100) min 245 nm (ε 2000); (0.1 N NaOH) max 277 nm (ε 5000) min 251 nm (ε 3100). Anal. Calcd. for $C_7H_9IN_2O_4$: C, 26.95; H, 2.91; N, 8.98. Found: C, 27.02; H, 3.00; N, 8.84.

1-[(2-Acetoxyethoxy)methyl]-5-nitrouracil (70)

Under the conditions outlined in "coupling method A" 235.5 mg (1.5 mmol) of 5-nitrouracil were coupled with 197 mg (1 mmol) of (2-acetoxyethoxy)methyl bromide (61). The crude product was chromatographed on silica

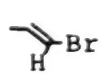
gel, with 5% MeOH/$CHCl_3$ as elutant, followed by crystallisation from $CHCl_3/Et_2O$ giving 236 mg (86%) of (70); mp 134-135°C; ^1H NMR (DMSO-d^6, 100 MHz) δ 1.99 (s, 3, CH_3), 3.79 and 4.11 (A_2B_2 multiplet, 2 and 2, OCH_2CH_2OAc), 5.27 (s, 2, NCH_2O), 9.29 (s, 1, H6), 12.0 (broad s, 1, NH). MS (CI) m/z 291 (M^+ + 18). UV (0.1 N HCl) max 296 and 234 nm (ε 9900 and 8000) min 257 and 206 nm (ε 3200 and 1900); (0.1 N NaOH) max 323 nm (ε 13,000) min 264 nm (ε 2700). Anal. Calcd. for $C_9H_{11}N_3O_7$: C, 39.57; H, 4.06; N, 15.38. Found: C, 39.31; H, 4.03; N, 15.22.

1-[(2-Hydroxyethoxy)methyl]-5-nitrouracil (86)

A 180 mg (0.659 mmol) sample of 1-[(2-acetoxyethoxy)methyl]-5-nitrouracil (70) was deacylated as described in "deprotection method B". The crude product was recrystallised from $MeOH/Et_2O$ to give 140 mg (92%) of (86); mp 173-175°C; ^1H NMR (DMSO-d^6, 100 MHz) δ 3.58 (multiplet, 4, OCH_2CH_2O), 5.70 (t, J = 5 Hz, 1, OH) 6.28 (s, 2, NCH_2O), 9.30 (s, 1, H6) 12.05 (broad s, 1, NH). MS m/z 231.0487. Calcd. for M^+: 231.0491. UV (0.1 N HCl) max 297 and 232 nm (ε 9900 and 7700) min 257 and 209 nm (ε 3100 and 4800); (0.1 N NaOH) max 323 nm (ε 13,300) min 263 nm (ε 2800). Anal. Calcd. for $C_7H_9N_3O_6$: C, 36,37; H, 3.92; N, 18.18.

Found: C, 36.24; H, 3.88; N, 18.21.

1-[(2-Acetoxyethoxy)methyl]-(E)-5-(2-bromovinyl)uracil (71)

Under the conditions outlined in "coupling method A" 325.5 mg (1.5 mmol) of (E)-5-(2-bromovinyl)uracil were coupled with 197 mg (1 mmol) of (2-acetoxyethoxy)-methyl bromide (61). The crude product was chromatographed on silica gel, with 2% MeOH/$CHCl_3$ as elutant, followed by crystallisation from $CHCl_3/Et_2O$ giving 289 mg (87%) of (71); mp 109-110°C; ^1H NMR (DMSO-d^6, 400 MHz) δ 1.99 (s, 3, CH_3), 3.72 and 4.10 (A_2B_2 multiplet, 2 and 2, OCH_2CH_2OAc), 5.12 (s, 2, NCH_2O), 6.85 (d, J = 13.5 Hz, 1,), 7.28 (d, J = 13.5 Hz,  , 8.00 (s, 1, H6), 9.5 (broad s, 1, NH). MS (CI) m/z 352, 350 ($M^+ + 18[^{79}Br,^{81}Br]$). UV (0.1 N HCl) max 289 and 249 nm (ε 11,300 and 16,400) min 270 and 214 nm (ε 8900 and 8300); (0.1 N NaOH) max 251 nm (ε 11,500) min 230 nm (ε 14,000) with shoulder at 280 nm (ε 9800). Anal. Calcd. for $C_{11}H_{13}BrN_2O_5$: C, 39.66; H, 3.93; N, 8.41. Found: C, 39.53; H, 4.02; N, 8.31.

(E)-5-(2-Bromovinyl)-1-[(2-hydroxyethoxy)methyl]-uracil (87)

A 500 mg (1.5 mmol) sample of 1-[(2-acetoxy-

ethoxy)methyl]-(E)-5-(2-bromovinyl)uracil (71) was deacylated as described in "deprotection method A". The crude product was recrystallised from acetone/pentane to give 398 mg (91%) of (87); mp 130-133°C; ^{1}H NMR (DMSO-d^6, 100 MHz) δ 3.50 (s, 4, OCH_2CH_2O), 4.60 (broad s, 1, OH), 5.10 (s, 2, NCH_2O), 6.83 (d, J = 14 Hz, 1,), 7.28 (d, J = 14 Hz, 1,), 7.97 (s, 1, H6), 11.5 (broad s, 1, NH). MS (CI) m/z 308, 310 (M^+ + 18[^{79}Br,^{81}Br]). UV (0.1 N HCl) max 290 and 249 nm (ε 10,300 and 14,900) min 270 and 213 nm (ε 7900 and 6600); (0.1 N NaOH) max 253 nm (ε 15,300) min 230 nm (ε 12,500) with shoulder at 280 nm (ε 8900). Anal. Calcd. for $C_9H_{11}BrN_2O_4$: C, 37.13; H, 3.81; N, 9.63. Found: C, 37.04; H, 3.87; N, 9.51.

1-[(2-Acetoxyethoxy)methyl]-2-thiouracil (72)

Under the conditions outlined in "coupling method A" 1.536 g (12 mmol) of 2-thiouracil were coupled with 1.576 g (8 mmol) of (2-acetoxyethoxy)methyl bromide (61). The crude product was chromatographed on silica gel, with 2% MeOH/$CHCl_3$ as elutant, followed by crystallisation from $CHCl_3$/Et_2O to give 1.626 g (83%) of (72); mp 179-182°C; ^{1}H NMR (DMSO-d^6, 100 MHz) δ 2.02 (s, 3, CH_3), 3.75 and 4.15 (A_2B_2 multiplet, 2 and 2, OCH_2CH_2OAc), 5.47 (s, 2, NCH_2O), 6.20 (d, J = 7.5 Hz,

1, H5), 7.97 (d, J = 7.5 Hz, 1, H6), 10.72 (s, 1, NH). MS m/z 244.0495. Calcd. for M^+: 244.0518. UV (0.1 N HCl) max 274 nm (ε 15,600) min 241 nm (ε 3500); (0.1 N NaOH) max 266 nm (ε 12,000) min 256 nm (ε 11,600). Anal. Calcd. for $C_9H_{12}N_2O_4S$: C, 44.26; H, 4.95; N, 11.47. Found: C, 44.17; H, 4.91; N, 11.50.

1-(Methoxymethyl)uracil (73)

Under the conditions outlined in "coupling method A" 246 mg (2.2 mmol) of uracil (5) were coupled with 61 mg (2 mmol) of chloromethylmethyl ether (63). The crude product was chromatographed on silica gel, with 2% MeOH/$CHCl_3$ as elutant, followed by crystallisation from $CHCl_3/Et_2O$ giving 269 mg (86%) of (73); mp 158-159°C; ^1H NMR (DMSO-d^6, 100 MHz) δ 3.26 (s, 3, OCH_3), 5.02 (s, 2, NCH_2O), 5.61 (d, J = 8 Hz, 1, H5), 7.69 (d, J = 8 Hz, 1, H6), 11.30 (s, 1, NH). MS m/z 156.0535. Calcd. for M^+: 156.0535. UV (0.1 N HCl) max 258 nm (ε 9400) min 228 nm (ε 1800); (0.1 N NaOH) max 259 nm (ε 6700) min 242 nm (ε 5600). Anal. Calcd. for $C_6H_8N_2O_3$: C, 46.15, H, 5.16; N, 17.94. Found: C, 45.96; H, 5.22; N, 17.86.

1-(Methoxymethyl)thymine (74)

Under the conditions outlined in "coupling method

A" 277 mg (2.2 mmol) of thymine were coupled with 161 mg (2 mmol) of chloromethylmethyl ether (63). The crude product was chromatographed on silica gel, with 2% MeOH/$CHCl_3$ as elutant, followed by crystallisation from $CHCl_3/Et_2O$ to give 265 mg (78%) of (74); mp 138-140; ^1H NMR (DMSO-d^6, 100 MHz) δ 1.78 (d, J = 1 Hz, 3, C5-CH_3), 3.25 (s, 3, OCH_3), 4.99 (s, 2, NCH_2O), 7.56 (q, J = 1 Hz, 1, H6), 11.30 (s, 1, NH). MS m/z 170.0691. Calcd. for M^+: 170.0691. UV (0.1 N HCl) max 265 nm (ε 8700) min 234 nm (ε 1300) (0.1 N NaOH) max 264 nm (ε 6400) min 243 nm (ε 3900). Anal. Calcd. for $C_7H_{10}N_2O_3$: C, 49.41; H, 5.92; N, 16.46. Found: C, 49.23; H, 5.96; N, 16.43.

1-(Methoxymethyl)-5-nitrouracil (75)

Under the conditions outlined in "coupling method A" 170 mg (1.08 mmol) of 5-nitrouracil were coupled with 80.5 mg (1 mmol) of chloromethymethyl ether (63). The crude product was chromatographed on silica gel, with 2% MeOH/$CHCl_3$ as elutant, followed by crystallisation from 95% EtOH to give 145 mg (72%) of (75); mp 148-150°C; ^1H NMR (DMSO-d^6, 100 MHz) δ 3.34 (s, 3, OCH_3) 5.19 (s, 2, NCH_2O) 9.28 (s, 1, H6) 12.06 (broad s, 1, NH). MS m/z 201.0385. Calcd. for M^+: 201.0390. UV (0.1 N HCl) max 297 and 236 nm (ε 10,400

and 8300) min 258 and 214 nm (ε3500 and 5300); (0.1 N NaOH) max 318 nm (ε 14,400) min 252 nm (ε 3800). Anal. Calcd. for $C_6H_7N_3O_5$: C, 35.83; H, 3.51; N, 20.89. Found: C, 35.84; H, 3.55; N, 20.59.

1-[(2-Acetoxyethoxy)methyl]cytosine (76)

Under the conditions outlined in "coupling method A" 167 mg (1.5 mmol) of cytosine were coupled with 197 mg (1 mmol) (2-acetoxyethoxy)methyl bromide (61). The crude product was chromatographed on silica gel, with 10% MeOH/$CHCl_3$ as elutant, followed by crystallisation from MeOH/Et_2O to give 193 mg (85%) of (76); mp 184-186°C; ^1H NMR (DMSO-d^6, 100 MHz) δ 1.98 (s, 3, CH_3), 3.66 and 4.05 (A_2B_2 multiplet, 2 and 2, OCH_2CH_2OAc), 5.06 (s, 2, NCH_2O), 5.68 (d, J = 7.5 Hz, 1, H5), 7.15 (broad s, 2, NH_2), 7.58 (d, J = 7.5 Hz, 1, H6). MS m/z 227.0907. Calcd. for M^+: 227.0907. UV (0.1 N HCl) max 275 nm (ε 12,000) min 240 nm (ε 1600); (0.1 N NaOH) max 266 nm (ε 7700) min 249 nm (ε 6200). Anal. Calcd. for $C_9H_{13}N_3O_4$: C, 47.58; H, 5.77; N, 18.49. Found: C, 47.43; H, 5.71; N, 18.42.

1-[(2-Hydroxyethoxy)methyl]cytosine (88)

A 2.905 g (12.8 mmol) sample of 1-[(2-acetoxyethoxy)methyl]cytosine (76) was deacylated as described

in "deprotection method B". The crude product was recrystallised from iPrOH/Et_2O to give 2.22 g (94%) of (88); mp 170-171°C; ^1H NMR (DMSO-d^6, 100 MHz) δ 3.46 (s, 4, OCH_2CH_2O), 4.50 (broad s, 1, OH), 5.06 (s, 2, NCH_2O), 5.72 (d, J = 7.5 Hz, 1, H5), 7.2 (broad s, 2, NH_2) 7.60 (d, J = 7.5 Hz, 1, H6). MS m/z 185.0803. Calcd. for M^+: 185.0800. UV (0.1 N HCl) max 276 nm (ε 12,000) min 240 nm (ε 1700) (0.1 N NaOH) max 267 nm (ε 7,900) min 250 nm (ε 6,400). Anal. Calcd. for $C_7H_{11}N_3O_3$: C, 45.40; H, 5.99; N, 22.69. Found: C, 45.37; H, 6.10; N, 22.66.

1-[(2-Acetoxyethoxy)methyl]-5-nitrocytosine (77)

Under the conditions outlined in "coupling method A" 468 mg (3 mmol) of 5-nitrocytosine (92) were coupled with 394 mg (2 mmol) of (2-acetoxyethoxy)methyl bromide (61). The crude product was chromatographed on silica gel, with 5% MeOH/$CHCl_3$ as elutant, followed by crystallisation from MeOH/Et_2O to give 407 mg (75%) of (77); mp 108-109°C; ^1H NMR (DMSO-d^6, 100 MHz) δ 2.01 (s, 3, CH_3), 3.57 and 4.10 (A_2B_2 multiplet, 2 and 2, OCH_2CH_2OAc), 5.29 (s, 2, NCH_2O), 8.06 (s, 1, NH), 8.56 (s, 1, NH), 9.27 (s, 1, H6). MS m/z 273.0844. Calcd. for MH^+: 273.0835. UV (0.1 N HCl) max 307 and 253 nm (ε 9200 and 8500) min 273 and 230 nm (ε 3500 and 4600);

(0.1 N NaOH) max 334 nm (ε 17,100) min 255 nm (ε 2900). Anal. Calcd. for $C_9H_{12}N_4O_6$: C, 39.71; H, 4.44; N, 20.58. Found: C, 39.43; H, 4.37; N, 20.30.

1-[(2-Hydroxyethoxy)methyl]-5-nitrocytosine (89)

A 220 mg (0.81 mmol) sample of 1-[(2-acetoxyethoxy)methyl]-5-nitrocytosine (77) was deacylated as described in "deprotection method B". The crude product was recrystallised from MeOH to give 170 mg (91%) of (89); mp 192-193°C; ^{1}H NMR (DMSO-d^6, 100 MHz) δ 3.54 (multiplet, 2, OCH_2CH_2O), 4.66 (t, J = 6 Hz, 1, OH), 5.26 (s, 2, NCH_2O), 8.04 (s, 1, NH), 8.53 (s, 1, NH), 9.26 (s, 1, H6). MS m/z 231.0737. Calcd. for MH^+: 231.0730. UV (0.1 N HCl) max 308 and 253 nm (ε 9000 and 8600) min 273 and 232 nm (ε 3600 an 4700) (0.1 N NaOH) max 335 nm (ε 17,200) min 255 nm (ε 2800). Anal. Calcd. for $C_7H_{10}N_4O_5$: C, 36.53; H, 4.38; N, 24.34. Found: C, 36.26; H, 4.36; N, 24.11.

1-[(2-Acetoxyethoxy)methyl]isocytosine (78)

Under the conditions outlined in "coupling method A" 760 mg (6.85 mmol) of isocytosine were coupled with 1.228 (6.2 mmol) of (2-acetoxyethoxy)methyl bromide (61). The crude product was chromatographed on silica gel, with 5% MeOH/$CHCl_3$ as elutant, followed by

crystallisation from MeOH/Et_2O gave 1.189 g (87%) of (78); mp 132-133°C; ^1H NMR (DMSO-d^6, 100 MHz) δ 2.00 (s, 3, CH_3), 3.75 and 4.10 (A_2B_2 multiplet, 2 and 2, OCH_2CH_2OAc), 5.17 (s, 2, NCH_2O), 5.96 (d, J = 8 Hz, 1, H5), 7.86 (d, J = 8 Hz, 1, H6), 8.20 (broad s, 2, NH_2). MS m/z 227.0902. Calcd. for M^+: 227.0906. UV (0.1 N HCl) max 255 nm (ε 7500) min 236 nm (ε 4700); (0.1 N NaOH) max 252 nm (ε 5100). Anal. Calcd. for $C_9H_{13}N_3O_4.\frac{1}{2}H_2O$: C, 45.76; H, 5.97; N, 17.79. Found: C, 45.81; H, 5.76; N, 17.84.

1-[(2-Hydroxyethoxy)methyl]isocytosine (90)

A 800 mg (3.5 mmol) sample of 1-[(2-acetoxyethoxy)-methyl]isocytosine (78) was deacylated as described in "deprotection method A". The crude product was recrystallised from MeOH to give 625 mg (96%) of (90); mp 187-189; ^1H NMR (DMSO-d^6, 100 MHz) δ 3.51 (s, 4, OCH_2CH_2O), 4.80 (s, 1, OH), 5.13 (s, 2, NCH_2O), 5.56 (d, J = 8 Hz, 1, H5), 6.95 (s, 2, NH_2), 6.48 (d, J = 8 Hz, 1, H6). MS m/z 185.0794. Calcd. for M^+: 185.0800. UV (0.1 N HCl) max 255 and 216 nm (ε 7900 and 9700) min 237 and 210 nm (ε 5400 and 9400) (0.1 N NaOH) max 250 nm (ε 6100). Anal. Calcd. for $C_7H_{11}N_3O_3.\frac{1}{4}H_2O$: C, 44.32; H, 6.11 N, 22.15. Found: C, 44.43; H, 5.97; N, 22.34.

1-[(2-Acetoxyethoxy)methyl]-3-deazauracil (79).

Under the conditions outlined in "coupling method A" 122 mg (1.1 mmol) of 3-deazauracil were coupled with 197 mg (1 mmol) of (2-acetoxyethoxy)methyl bromide (61). The crude product was chromatographed on silica gel, with 2% MeOH/$CHCl_3$ elutant, followed by crystallisation from iPrOH/Et_2O to give 200 mg (88%) of (79); mp 123-124°C; ^1H NMR (DMSO-d^6, 200 MHz) δ 2.00 (s, 3, CH_3), 3.66 and 4.08 (A_2B_2 multiplet, 2 and 2, OCH_2CH_2OAc), 5.21 (s, 2, NCH_2O), 5.59 (d, J = 2.4 Hz, 1, H3), 5.90 (d of d, J = 2.4 Hz, J = 7.6 Hz, 1, H5), 7.54 (d, J = 7.6 Hz, 1, H6), 10.8 (broad s, 1, OH). MS m/z 227.0795. Calcd. for M^+: 227.0785. UV (0.1 N HCl) max 280 nm (ε 4600) min 247 nm (ε 700); (0.1 N NaOH) max 256 nm (ε 6800) min 232 nm (ε 3700). Anal. Calcd. for $C_{10}H_{13}NO_5$: C, 52.86; H, 5.77; N, 6.16. Found: C, 52.87; H, 5.76; N, 6.15.

1-[(2-Hydroxyethoxy)methyl]-3-deazauracil (91)

A 250 mg (1.1 mmol) sample of 1-[(2-acetoxyethoxy)-methyl]-3-deazauracil (79) was deacylated as described in "deprotection method B". The crude product was recrystallised from iPrOH/Et_2O to give 169 mg (83%) of (91); mp 137-139°C; ^1H NMR (DMSO-d^6, 100 MHz) δ 3.46 (s, 4, OCH_2CH_2O), 4.55 (broad s, 1, OH) 5.18 (s, 2,

NCH_2O), 5.56 (d, J = 2.5 Hz, 1, H3), 5.87 (d of d, J = 2.5 Hz, J = 7.5 Hz, 1, H5), 7.52 (d, J = 7.5 Hz, 1, H6). MS m/z 185.0689. Calcd. for M^+: 185.0688. UV (0.1 N HCl) max 280 nm (ε 4600) min 247 nm (ε 800); (0.1 N NaOH) max 256 nm (ε 6900) min 232 nm (ε 3700). Anal. Calcd. for $C_8N_{11}NO_4$: C, 51.89; H, 5.99; N, 7.56. Found: C, 51.80; H, 6.00; N, 7.53.

5-Chloro-1-[(2-hydroxyethoxy)methyl]cytosine (93)

To a solution of 185 mg (1 mmol) of 1-[(2-hydroxyethoxy)methyl]cytosine (88) in 0.5 m HCl/dry DMF was slowly added 2 mL of DMF containing 300 mg (1.74 mmol) of m-chloroperbenzoic acid. The solution was allowed to stir at room temperature for 2 h at which time a additional 70 mg of m-chloroperbenzoic acid (in 1 mL DMF) and 4 mL of 0.5 m HCl/dry DMF were added. After an additional 2 h the solvent was removed in vacuo and the residue partitioned between H_2O and Et_2O. Neutralisation of the aqueous layer (dowex resin 1X2 OH^-), filtration, and removal of solvent left a crude solid which was recrystallised from 95% EtOH to give 180 mg (82%) of (93); mp 177.5-179°C; ^1H NMR (DMSO-d^6, 200 MHz) δ 3.50 (multiplet, 4, OCH_2CH_2O, 4.65 (t, J = 5 Hz, 1, OH), 5.08 (s, 2, NCH_2O) 7.26 and 7.89 (s and s, 1, and 1, NH_2), 8.05 (s, 1, H6). MS m/z 219.0410,

221.0381 (^{35}Cl,^{37}Cl). Calcd. for M^+: 219.0410, 221.0381 (^{35}Cl,^{37}Cl). UV (0.1 N HCl) max 293 nm (ε 9600) min 253 nm (ε 1100) (0.1 N NaOH) max 283 nm (ε 6300) min 260 nm (ε 3600). Anal. Calcd. for $C_7H_{10}ClN_3O_3$: C, 38.28, H, 4.59; N, 19.13; Cl, 16.14. Found: C, 38.14; H, 4.58; N, 19.06; Cl, 16.09.

5-Bromo-1-[(2-hydroxyethoxy)methyl]cytosine (94)

A 185 mg (1 mmol) of 1[(2-hydroxyethoxy)methyl]-cytosine (88) was brominated by the same procedure described above for the synthesis of (93) except that 0.5 M HBr/dry DMF was used in the place of 0.5 M HCl/dry DMF. The crude product was chromatographed on silica gel (25 g, 20% EtOH/$CHCl_3$) and then crystallised from 95% EtOH to give 122 mg (46%) of (94); mp 173-174°C; ^1H NMR (DMSO-d^6, 200 MHz) δ 3.50 (multiplet, 4, OCH_2CH_2O), 4.66 (t, J = 5 Hz, 1, OH), 5.09 (s, 1, NCH_2O), 7.06 and 7.91 (s and s, 1 and 1, NH_2), 8.12 (s, 1, H6). MS m/z 265.9957, 263.9969 (^{81}Br,^{79}Br). Calcd. for M^+: 265.9963, 263.9983. UV (0.1 N HCl) max 295 nm (ε 9800) min 253 nm (ε 1000); (0.1 N NaOH) max 284 nm (ε 6400) min 260 nm (ε 3800). Anal. Calcd. for $C_7H_{11}BrN_3O_3$: C, 31.84; H, 3.82; N, 15.91; Br, 30.26. Found: C, 32.12; H, 4.01; N, 15.70; Br, 30.17.

1-[(2-Acetoxyethoxy)methyl]pyrimidin-4-one (95)

A 880 mg (3.6 mmol) sample of 1-[(2-acetoxyethoxy)-methyl]-2-thiouracil (72) was dissolved in a suspension of rainey-nickel (400 mg) in 95% EtOH (25 mL). The reaction was allowed to stir at room temperature for 18 h at which point the mixture was filtered. Removal of solvent from the filtrate left crude (95) which was purified by preparative thin layer chromatography and crystallisation from $CHCl_3/Et_2O$ to give 428 mg (56%) of (95); mp 107-108°C; ^1H NMR (DMSO-d^6, 100 MHz) δ 2.00 (s, 3, CH_3), 3.70 and 4.12 (A_2B_2 multiplet, 2 and 2, OCH_2CH_2O), 5.24 (s, 2, NCH_2O), 6.06 (d, J = 8 Hz, 1, H5), 7.85 (d of d, J = 3 Hz, J = 8 Hz, 1, H6) 8.48 (d, J = 3 Hz, 1, H2). MS m/z 212.0799. Calcd. for M^+: 212.0797. UV (0.1 N HCl) max 232 nm (ε 11,800) min 209 nm (ε 4200); (0.1 N NaOH) max 240 nm (ε 13,400) min 223 nm (ε 7200). Anal. Calcd. for $C_9H_{12}N_2O_4$: C, 50.94; H, 5.70; N, 13.20. Found: C, 50.76; H, 5.73; N, 13.01.

Deprotection method C

1-[(2-Hydroxyethoxy)methyl]pyrimidin-4-one (96)

A 200 mg (0.94 mmol) sample of 1-[(2-acetoxy-ethoxy)methyl]pyrimidin-4-one (95) was dissolved in a solution of $MeOH/H_2O/NEt_3$ (1:1:2.5) and allowed to stir

at room temperature for 6 h. Removal of volatiles followed by recrystallisation from iPrOH gave 134 mg (84%) of (96); mp 126-129°C; ^{1}H NMR (DMSO-d^6, 100 MHz) δ 3.49 (s, 4, OCH_2CH_2O), 4.74 (broad s, 1, OH), 5.22 (s, 2, NCH_2O), 6.05 (d, J = 7.5 Hz, 1, H5), 7.82 (d of d, J = 3 Hz, J = 7.5 Hz, 1, H6), 8.44 (d, J = 3 Hz, 1, H2). MS m/z 170.0690. Calcd. for M^+: 170.0692. UV (0.1 N HCl) max 225 nm (ε 9700) min 199 nm (ε 3700); (0.1 N NaOH) max 230 nm (ε 9200) min 213 (ε 4900) with shoulder at 249 nm (ε 8700). Anal. Calcd. for $C_7H_{10}N_2O_3$: C, 49.41; H, 5.92; N, 16.46. Found: C, 49.17; H, 5.87; N, 16.39.

3-[(2-Acetoxyethoxy)methyl]cytosine (98)

To a stirred suspension of 1.22 g (11 mmol) of cytosine (3) in 10 mL of dry DMF was slowly added 1.97 g (10 mmol) of (2-acetoxyethoxy)methyl bromide (61). The resulting solution was allowed to stir at room temperature for 3 h after which the solvent was removed in vacuo and the residual oil applied to a silica gel column (200 g, elutant 10% MeOH/$CHCl_3$). The first eluted product was 1,3-bis-[(2-acetoxyethoxy)-methyl]cytosine (99); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.09 and 2.10 (s, s, 1 and 1, $COCH_3$, $COCH_3$'), 3.77 and 3.88 (A_2B_2 multiplet, 2 and 2, OCH_2CH_2OAc), 4.21 (A_2B_2

multiplet, 4, $OCH_2\underline{CH_2}OAc$), 5.12 and 5.58 (s, s, 1 and 1, NCH_2O, $NCH_2'O$), 5.69 (d, J = 8 Hz, 1, H5), 6.34 (broad s, 1, NH), 6.79 (d, J = 8 Hz, 1, H6). MS m/z 343.1371. Calcd. for M^+: 343.1380. UV (0.1 N HCl) max 280 nm (ε 8000) min 242 nm (ε 1200); (0.1 N NaOH) max 267 nm (ε 7300) max 241 nm (ε 3900). However this compound could only be crystallised satisfactorily after deacylation as outlined in "Deprotection method C". Good crystals of 1,3-bis[(2-hydroxyethoxy)-methyl]cytosine (101) were obtained from $MeOH/Et_2O$. Mp 102-104°C; ^1H NMR (DMSO-d^6, 100 MHz) δ 3.48 (multiplet, 8, $OCH_2\underline{CH_2}O$'s) 4.5 (v broad, 2, OH's), 5.02 and 5.38 (s and s, 1 and 1, NCH_2O, $NCH_2'O$), 5.76 (d, J = 8 Hz, 1, H5), 6.50 (v broad, 1, NH), 7.10 (d, J = 8 Hz, 1, H6). MS m/z 260.1240. Calcd. for M^+: 260.1263. UV (0.1 N HCl) max 277 nm (ε 8800) min 240 nm (ε 1400); (0.1 N NaOH) max 266 nm (ε 8200) min 241 nm (ε 4400). Anal. Calcd. for $C_{10}H_{18}N_3O_5$: C, 46.32; H, 6.61; N, 16.21. Found: C, 46.00, H, 6.62; N, 15.85.

The second eluted product was 3-[(2-acetoxy-ethoxy)methyl]cytosine (98). The solvent was removed from appropriate fractions and the crude product was crystallised from $CHCl_3/Et_2O$ to give 730 mg (32%) of (98); mp 156-158°C; ^1H NMR (DMSO-d^6, 100 MHz) δ 2.00 (s, 3, CH_3), 3.82 and 4.23 (A_2B_2 multiplet, 2

and 2, OCH_2CH_2OAc), 5.47 (s, 2, NCH_2O) 6.14 (d, J = 8 Hz, 1, H5), 7.84 (d, J = 8 Hz, 1, H6), 10.5 (broad, 2, NH_2). MS m/z 228.0978. Calcd. for M^+: 228.0984. UV (0.1 N HCl) max 278 nm (ε 9800) min 235 nm (δ 1400); (0.1 N NaOH) max 301 nm (ε 7100) min 253 nm (ε 700). Anal. Calcd. for $C_9H_{13}N_3O_4$: C, 47.58; H, 5.77; N, 18.49. Found: C, 47.29; H, 5.75; N, 18.33.

3-[(2-Hydroxyethoxy)methyl]cytosine (100)

A 350 mg (1.54 mmol) sample of 3-[(2-acetoxy-ethoxy)methyl]cytosine (98) was deacylated as outlined in "Deprotection method C". Recrystallisation from MeOH gave 237 mg (83%) of (100); mp 137-139°C; ^1H NMR (DMSO-d^6, 200 MHz) δ 3.51 and 3.60 (A_2B_2 multiplet, 2 and 2, OCH_2CH_2O), 5.44 (s, 2, NCH_2O), 6.06 (d, J = 7 Hz, 1, H5) 7.78 (d, J = 7 Hz, 1, H6), 9.5 (v broad, 2, NH_2). MS m/z No M^+ peak found: 140.0459 (3% $B\text{-}\overset{\oplus}{C}H_2O$), 124.0512 (3% $B^{\oplus}=CH_2$), 111.0432 (100% $B^{\oplus}$), 75.0450 (12% $HOCH_2CH_2O\overset{\oplus}{C}H_2$). UV (0.1 N HCl) max 279 nm (ε 9800) min 240 nm (ε 800); (0.1 N NaOH) max 302 nm (ε 9900) min 256 (ε 1100). Anal. Calcd. for $C_7H_{11}N_3O_3$: C, 45.40; H, 5.99; N, 22.69. Found: C, 45.22; H, 6.04; N, 22.60.

Coupling Method B

9-[(2-Acetoxyethoxy)methyl]-2-amino-6-chloropurine (103)

A stirred mixture of 187 mg (1.1 mmol) of 2-amino-6-chloropurine, 45 mg of $(NH_4)_2SO_4$ and 5 mL of hexamethyldisilazane was heated at reflux for 3 h with exclusion of moisture. Volatile materials were evaporated *in vacuo* with protection against moisture. The residue was stirred with 15 mL of dry benzene and 344 mg (1.36 mmol) of $Hg(CN)_2$ was added. This mixture was stirred at reflux under a dry nitrogen atmosphere for 30 min. A solution of 197 mg (1 mmol) of (2-acetoxyethoxy)methyl bromide (61) in 5 mL of dry benzene was added and the reflux continued for 2 h. The mixture was cooled, 150 mL of $CHCl_3$ added and the organic phase was washed with 30 mL of saturated $NaHCO_3/H_2O$ followed by 30 mL of 1 M KI/H_2O. The organic solution was dried over Na_2SO_4, filtered and evaporated to give 239 mg (84%) of an oil that crystallised from $CHCl_3/Et_2O$ to give pure (103); mp 132-133°C; ^1H NMR (DMSO-d^6, 100 MHz) δ 2.03 (s, 3, CH_3), 3.75 and 4.18 (A_2B_2 multiplet, 2 and 2, OCH_2CH_2OAc), 5.28 (broad s, 2, NH_2), 5.52 (s, 2, NCH_2O), 7.92 (s, 1, H8). MS m/z 285.0633, 287.0605 (^{35}Cl,^{37}Cl); calcd. for M^+: 285.0628, 287.0599 (^{35}Cl,^{37}Cl). UV (0.1 N HCl), max 309 and 246 nm

(ε 7800 and 6900), min 262 and 232 nm (ε 700 and 5000); (0.1 N NaOH) max 309 and 246 nm (ε 7800 and 6900) min 262 and 232 nm (ε 700 and 5000). Anal. Calcd. for $C_{10}H_{12}ClN_5O_3$: C, 42.04; H, 4.23; N, 24.51. Found: C, 41.81; H, 4.21; N, 24.56.

2-Amino-6-chloro-9-[(2-hydroxyethoxy)methyl]purine (108)

A 226 mg (0.79 mmol) sample of 9-[(2-acetoxyethoxy)methyl]-2-amino-6-chloropurine (103) was deacylated as described in "deprotection method A". The crude product was recrystallised from iPrOH to give 168 mg (87%) of (108); mp 204-205°C; ^1H NMR (DMSO-d^6, 100 MHz) δ 3.50 (s, 4, OCH_2CH_2O), 4.66 broad s, 1, OH), 4.57 (s, 2, NCH_2O), 6.99 (broad s, 2, NH_2), 8.28 (s, 1, H8). MS m/z 243.0520, 245.0494 (^{35}Cl,^{37}Cl). Calcd. for M^+: 243.0523, 245.0494 (^{35}Cl,^{37}Cl). UV (0.1 N HCl) max 307 and 246 nm (ε 7600 and 7200) min 264 and 232 nm (ε 1000 and 5300) (0.1 N NaOH) max 307 and 246 nm (ε 7600 and 7200) min 265 and 232 nm (ε 1100 and 5400). Anal. Calcd. for $C_8H_{10}ClN_5O_2$: C, 39.44; H, 4.14; N, 28.74. Found: C, 39.25; H, 4.12; N, 28.54.

9-[(2-Acetoxyethoxy)methyl]-2,6-dichloropurine (104)

Under the conditions outlined in "coupling method B"

208 mg (1.1 mmol) of 2,6-dichloropurine were coupled with 197 mg (1 mmol) of (2-acetoxyethoxy)methyl bromide (61) to give 270 mg (89%) of (104), which was crystallised from $CHCl_3/Et_2O$; mp 96-97°C; ^1H NMR (DMSO-d^6, 100 MHz) δ 2.03 (s, 3, CH_3), 3.80 and 4.20 (A_2B_2 multiplet, 2 and 2, OCH_2CH_2OAc), 5.68 (s, 2, NCH_2O), 8.27 (s, 1, H8). MS m/z 306.0061, 304.0124 ($^{37}Cl^{35}Cl$, $^{35}Cl^{35}Cl$). Calcd. for M^+: 306.0100, 304.0130 ($^{37}Cl^{35}Cl$, $^{35}Cl^{35}Cl$). UV (0.1 N HCl) max 273 nm (ε 9,400) min 228 nm (ε 2400) with shoulder at 250 nm (ε 4800); (0.1 N NaOH) max 274 nm (ε 8900) min 233 nm (ε 2800) with shoulder at 250 nm (ε 4800). Anal. Calcd. for $C_{10}H_{10}Cl_2N_4O_3$: C, 39.36; H, 3.30; N, 18.36. Found: C, 39.18; H, 3.44; N, 18.22.

9-[(2-Acetoxyethoxy)methyl]-6-chloropurine (102)

Under the conditions outlined in "coupling method B" 170 mg (1.1 mmol) of 6-chloropurine were coupled with 197 mg (1 mmol) of (2-acetoxyethoxy)methyl bromide (61) to give 224 mg (83%) of (102) which was crystallised from CH_2Cl_2/Et_2O; mp 96-97°C; ^1H NMR (DMSO-d^6, 100 MHz) δ 1.91 (s, 3, CH_3), 3.76 and 4.06 (A_2B_2 multiplet, 2 and 2, OCH_2CH_2OAc), 5.74 (s, 2, NCH_2O), 8.84 and 8.86 (s, s, 1 and 1, H2, H8). MS (CI) m/z 271, 273 ($MH^+[^{35}Cl,^{37}Cl]$). UV (0.1 N HCl) max 263 nm

(ε 9100) min 222 nm (ε 2100) with shoulder at 250 nm (ε 6300); (0.1 N NaOH) max 263 nm (ε 8800) min 221 nm (ε 3300) with shoulder at 250 nm (ε 6300). Anal. Calcd. for $C_{10}H_{11}ClN_4O_3$: C, 44.37; H, 4.09; N, 20.70. Found: C, 44.02; H, 4.04; N, 20.78.

9-[(2-Hydroxyethoxy)methyl]guanine (22)

Method A.

From 2-amino-6-chloro-9-[(2-hydroxyethoxy)-methyl]purine (108)

To a solution of 110 mg (0.45 mmol) of 9-[(2-hydroxyethoxy)methyl]-2-amino-6-chloropurine (108) in 25 mL of aqueous phosphate buffer (0.05 M, pH 7.5) was added 30 mg of adenosine deaminase (adenosine amino-hydrolase, E.C. 3.5.4.4. Sigma type I, Sigma Chemical Co.). The reaction mixture was stirred at room temperature and monitored by T.L.C. (S.S.E.) until complete hydrolytic dechlorination had occurred. The crude product could be obtained directly by crystal-lisation from the concentrated reaction mixture. However a convenient purification procedure employed adsorption of the product from the aqueous medium on a carbon column, washing the column with water and eluting the desired product with 35% CH_3CN/H_2O. Evaporation of appropriate fractions, recrystallisation

from EtOH/H_2O and thorough drying of the product gave 88 mg (87%) of pure (22); mp 265-266°C; ^1H NMR (DMSO d^6, 400 MHz) δ 3.34 multiplet, 4, OCH_2CH_2O), 4.56 (s, 1, OH), 5.34 (s, 2, NCH_2O), 6.49 (broad s, 2, NH_2), 7.81 (s, 1, H8), 10.61 (s, 1, NH). MS m/z 225.0863. Calcd. for M^+: 225.0862. UV (0.1 N HCl) max 252 nm (ε 13,000) min 224 nm (ε 2400) with shoulder at 271 nm (ε 8800); (0.1 N NaOH) max 260 nm (ε 11,400) min 229 (ε 4700). Anal. Calcd. for $C_8H_{11}N_5O_3$: C, 42.67; H, 4.92; N, 31.10. Found: C, 42.45; H, 4.93; N, 30.95.

Method B.

From 9-[(2-acetoxyethoxy)methyl]-2,6-dichloropurine (104)

A solution of 510 mg (1.67 mmol) of 9-[(2-acetoxyethoxy)methyl]-2,6-dichloropurine (104) in 15 mL of liquid ammonia was sealed in a parr steel bomb and heated at 150°C for 3 days. After cooling, the bomb was vented and after evaporation of ammonia was complete the residue was applied to a silica column with 10% MeOH/$CHCl_3$ as elutant. Removal of solvent from appropriate fractions gave 350 mg (93%) of 2,6-diamino-9-[(2-hydroxyethoxy)methyl]purine (107) as an oil. This material was treated with adenosine deaminase as described above in Method A ((108) → (22))

to give 312 mg (89%) of (22).

Method C.

Overall from 2-amino-6-chloropurine

187 mg (1.1 mmol) of 2-amino-6-chloropurine were coupled with 197 mg (1 mmol) of (2-acetoxyethoxy)methyl bromide (61) as described in "coupling method B". Deprotection, by "deprotection method A", gave a crude solid of (108) which was subjected to adenosine deaminase as described previously. The resulting product was purified on a carbon column and was recrystallised to give 172 mg (76% overall yield) of (22).

Coupling Method C

9-[(2-Acetoxyethoxy)methyl]adenine (110)

To a stirred solution of 149 mg (1.1 mmol) of adenine (1) in 25 mL of dry (and amine-free) DMF was added 29 mg (1.2 mmol) of NaH. After evolution of hydrogen had ceased the mixture was cooled to -63°C and a solution of 197 mg (1 mmol) of (2-acetoxyethoxy)-methyl] bromide (61) in 5 mL of DMF was added slowly. The stirred mixture was allowed to warm slowly to room temperature over a period of 2 h at which time 0.5 mL

of 1 M $NaHCO_3/H_2O$ was added and volatile materials were removed in vacuo. The residue was chromatographed on a column of silica gel using 5% MeOH/$CHCl_3$ as elutant. Appropriate fractions were combined and evaporated to give 188 mg (75%) of (110). This was recrystallised from $CHCl_3/Et_2O$: mp 156-158°C; ^1H NMR (DMSO-d^6, 100 MHz) δ 1.92 (s, 3, CH_3), 3.72 and 4.06 (A_2B_2 multiplet, 2 and 2, OCH_2CH_2OAc), 5.56 (s, 2, NCH_2O), 7.23 (s, 2, NH_2), 8.16 (s, 1, H2), 8.26 (s, 1, H8), MS m/z 251.1020. Calcd. for M^+: 251.1059. UV (0.1 N HCl) max 256 nm (ε 16,600), min 225 nm (ε 2400); (0.1 N NaOH) max 260 nm (ε 16,900) min 227 nm (ε 3300). Anal. Calcd. for $C_{10}H_{13}N_5O_3$: C, 47.81; H, 5.22; N, 27.87. Found: C, 47.51; H, 5.05; N, 28.01.

9-[(2-Hydroxyethoxy)methyl]adenine (23)

A 830 mg (3.3 mmol) sample of 9-[(2-acetoxyethoxy)methyl]adenine (110) was deacylated as described in "deprotection method B". The crude product was recrystallised from MeOH to give 648 mg (94%) of (23); mp 204-206°C, Lit. 199.5-200°C[297]; ^1H NMR (DMSO-d^6, 100 MHz) δ 3.50 (s, 4, OCH_2CH_2O) 4.65 (broad s, 1, OH), 5.56 (s, 2, NCH_2O), 7.24 (s, 2, NH_2), 8.17 (s, 1, H2), 8.26 (s, 1, H8). MS m/z 209.0910. Calcd. for M^+: 209.0913. UV (0.1 N HCl) max 256 nm (ε 14,200) min 225

nm (ε 2,300); (0.1 N NaOH) max 259 nm (ε 14,500) min 227 nm (ε 3200). Anal. Calcd. for $C_8H_{11}N_5O_2$: C, 45.93; H, 5.30; N, 33.48. Found: C, 45.69; H, 5.29; N, 33.24.

9-[(2-Hydroxyethoxy)methyl]hypoxanthine (111)

To a solution of 100 mg (0.48 mmol) of 9-[(2-hydroxyethoxy)methyl]adenine (23) in 30 mL of aqueous phosphate buffer (0.05 M, pH 7.5) was added 30 mg of adenosine deaminase (adenosine aminohydrolase, E.C.3.5.4.4. Sigma type I, Sigma Chemical Co.). The reaction mixture was stirred at room temperature and monitored by TLC (SSE) until complete deamination had occurred. The product was adsorbed onto a carbon column (10 g, Au-4). The column was washed with water and the desired product eluted with 50% CH_3CN/H_2O. Recrystallisation from 98% EtOH gave 88 mg (88%) of (111); mp 217-219°C; ^1H NMR (DMSO-d^6, 100 MHz) δ 3.51 (s, 4, OCH_2CH_2O) 5.57 (s, 2, NCH_2O), 8.07 (s, 1, H2), 8.21 (s, 1, H8). MS m/z 210.0761. Calcd. for M^+: 210.0753. UV (0.1 N HCl) max 248 nm (ε 12,400) min 217 nm (ε 2600); (0.1 N NaOH) max 252 nm (ε 13,200) min 225 nm (ε 3700). Anal. Calcd. for $C_8H_{10}N_4O_3$: C, 45.71; H, 4.80; N, 26.66. Found: C, 45.43; H, 4.81; N, 26.48.

9-[(2-Hydroxyethoxy)methyl]-N^6,N^6-dimethyladenine (106)

A 2.5 g (9.24 mmol) sample of 9-[(2-acetoxyethoxy)methyl]-6-chloropurine (102) was stirred in 150 mL of 40% methanolic dimethylamine at room temperature for 8 h. Removal of volatiles in vacuo followed by crystallisation of the crude product from iPrOH gave 1.826 g (83%) of (106); mp 107-108°C; ^{1}H NMR (DMSO-d^6, 100 MHz) δ 3.46 (s, 6, $N(CH_3)_2$), 3.51 (s, 4, OCH_2CH_2O), 5.66 (s, 1, OH) 5.59 (s, 2, NCH_2O), 8.26, 8.30 (s, s, 1 and 1, H2, H8). MS m/z 237.1222. Calcd. for M^+: 237.1225. UV (0.1 N HCl) max 267 nm (ε 18,800) min 230 nm (ε 1900); (0.1 N NaOH) max 274 nm (ε 19,500) min 235 nm (ε 2800). Anal. Calcd. for $C_{10}H_{15}N_5O_2$: C, 50.62; H, 6.37; N, 29.52. Found: C, 50.47; H, 6.34; N, 29.26.

9-[(2-Hydroxyethoxy)methyl]-N^6,N^6,N^3-trimethyladenine iodide (113)

A 1 g (4.2 mmol) sample of 9-[(2-hydroxyethoxy)-methyl]-N^6,N^6-dimethyladenine (106) was dissolved in 5 mL of dry DMF along with 2 mL (32 mmol) of methyl iodide and the solution was left to stand at 0°C for 3 weeks. Filtration, followed by washing of the crystals with acetone afforded 882 mg (55%) of (113); mp 238-242°C with decomposition; ^{1}H NMR (DMSO-d^6, 100 MHz)

δ 3.40, 3.87 (2 singlets, 6, $N(CH_3)_2$), 3.51 (s, 4, OCH_2CH_2O), 4.23 (s, 3, NCH_3), 5.91 (s, 2, NCH_2O), 8.61, 8.72 (s, s, 1 and 1, H2, H8). MS m/z (glycerol/FAB) 252 (M^+-I^-). UV (0.1 N HCl) max 286 nm (ε 18,200) min 247 nm (ε 3900). Anal. Calcd. for $C_{11}H_{18}IN_5O_2$: C, 34.84; H, 4.78; N, 18.47. Found: C, 34.83; H, 4.89; N, 18.47.

4-Carbamoyl-1-[(2-hydroxyethoxy)methyl]-5-methylamino-imidazole (115)

A 900 mg (2.37 mmol) sample of 9-[(2-hydroxy-ethoxy)methyl]-N^6,N^6,N^3-trimethyladenine iodide (113) was refluxed in 50% EtOH/2NKOH for 1 h. The solution was neutralised with amberlite resin (IR-120 $H^{\oplus}$), filtered and volatiles removed in vacuo. The residue was placed on a short silica gel column (25 g, elutant 20% EtOH in $CHCl_3$), appropriate fractions were collected and the solvent was removed. Recrystal-lisation was effected from 95% EtOH to give 418 mg (82%) of (115); mp 126-126°C; ^1H NMR (DMSO-d^6, 400 MHz) δ 2.92 (d, J = 6 Hz, 3, CH_3), 3.49 (multiplet, 4, OCH_2CH_2O) 4.70 (t, J = 5 Hz, 1, OH), 5.33 (s, 2, NCH_2O), 6.05 (q, J = 6 Hz, 1, $NHCH_3$), 6.79 and 6.95 (s and s, 1 and 1, $CONH_2$), 7.35 (s, 1, H2). MS m/z 214.1067. Calcd. for M^+: 214.1066. UV (0.1 N HCl)

max 256 nm (ε 8200) min 225 nm (ε 3300) with shoulder at 270 nm (ε 7600); (0.1 N NaOH) max 273 nm (ε 9400) min 228 nm (ε 5000). Anal. Calcd. for $C_8H_{14}N_4O_3$: C, 44.85; H, 6.59; N, 26.15. Found: C, 44.63; H, 6.65; N, 26.27.

5-Amino-4-carbamoyl-1-[(2-hydroxyethoxy)methyl]-imidazole (118)

To a stirred suspension of 460 mg (2 mmol) of 5-benzamido-4-carbamoylimidazole (116) in DMF (5 mL) containing 364 mg (4 mmol) of triethylamine was added 788 mg (4 mmol) of (2-acetoxyethoxy)methyl bromide (61). The solution was allowed to stir for 24 h at which time all volatiles were removed in vacuo and the residue was chromatographed on a silica gel column (100 g, 2% EtOH/$CHCl_3$). Removal of solvent from appropriate fractions gave 320 mg (46%) of 1-[(2-acetoxyethoxy)methyl]-5-benzamido-4-carbamoylimidazole (117) as an oil; ^1H NMR (DMSO-d^6, 100 MHz) δ 1.94 (s, 3, CH_3), 3.61 and 4.05 (A_2B_2 multiplet, 2 and 2, OCH_2CH_2OAc), 5.33 (s, 2, NCH_2O), 7.12 and 7.32 (s and s, 1 and 1, NH_2), 7.5-8.2 (multiplet, 6H, H2 and benzoyl protons), 10.26 (s, 1, NH). MS m/z 346.1281. Calcd. for M^+: 346.1277.

A 240 mg (0.9 mmol) sample of this oil (117) was

treated directly with hydrazine hydrate (3 mL) in refluxing 98% EtOH (10 mL). After 48 h volatiles were removed *in vacuo* and the residue was purified by preparative thin layer chromatography (silica gel, SSE). Crystallisation from EtOH/Et_2O gave 116 mg (84%) of (118); mp 136-137°C; ^1H NMR (DMSO-d^6, 200 MHz) δ 3.46 (multiplet, 4, OCH_2CH_2O), 4.70 (t, J = 5 Hz, 1, OH), 5.25 (s, 2, NCH_2O) 5.84 (s, 2, NH_2), 6.74 (broad s, 2, $CONH_2$), 7.24 (s, 1, H2). MS m/z 200.0908. Calcd. for M^+: 200.0908. UV (0.1 N HCl) max 267 and 243 nm (ε 10,700 and 9800) min 254 and 220 nm (ε 9100 and 4800); (0.1 N NaOH) max 266 nm (ε 12,700) min 226 nm (ε 6100). Anal. Calcd. for $C_7H_{12}N_4O_3$: C, 42.00; H, 6.04; N, 27.99. Found: C, 41.97; H, 6.03; N, 27.81.

8-Bromo-9-[(2-hydroxyethoxy)methyl]guanine (119)

To a stirred solution of 450 mg (2 mmol) of 9-[(2-hydroxyethoxy)methyl]guanine (22) in 100 mL H_2O was added bromine water slowly until the bromine colour persisted. The solution was allowed to stand for 2 h at 0°C, at which point the solid that separated was collected by filtration and recrystallised from EtOH/H_2O to give 507 mg (83%) of (119); decomposition starting at 280°C; ^1H NMR (DMSO-d^6, 100 MHz) δ 3.52 (multiplet, 4, OCH_2CH_2O), 4.68 (t, J = 5 Hz, 1, OH),

5.33 (s, 2, NCH_2O), 6.66 (s, 2, NH_2), 10.77 (s, 1, NH). MS (CI) m/z 304, 306 ($MH^{\oplus}[^{79}Br,^{81}Br]$). UV (0.1 N HCl) max 259 nm (ε 16,700) min 223 nm (ε 6400); (0.1 N NaOH) max 270 nm (ε 13,700) min 234 nm (ε 5700). Anal. Calcd. for $C_8H_{10}BrN_5O_3$: C, 31.60; H, 3.31; N, 23.03; Br, 26.28. Found: C, 31.69; H, 3.36; N, 23.06; Br, 26.35.

8-Chloro-9-[(2-hydroxyethoxy)methyl]guanine (120)

To a solution of 200 mg (0.89 mmol) of 9-[(2-hydroxyethoxy)methyl]guanine (22) in 2.5 mL of 0.5 M HCl/dry DMF was slowly added 1 mL of DMF containing 225 mg (1.3 mmol) of m-chloroperbenzoic acid. After stirring at room temperature for 2 min an additional 50 mg (0.29 mmol) of m-chloroperbenzoic acid in 0.5 mL of DMF was added, and the solution allowed to stir for 2 h. Saturated aqueous sodium bicarbonate solution was added to the reaction mixture dropwise until neutral pH was obtained. Volatiles were removed in vacuo and the residue was partitioned between H_2O and Et_2O. The residue from the aqueous layer, after removal of solvent, was further purified on a silica column (50 g, 20% EtOH/$CHCl_3$). Crystallisation from EtOH/H_2O gave 121 mg (52%) of (120); decomposition starting at 280°C; ^1H NMR (DMSO-d^6, 200 MHz) δ 3.49 (multiplet, 4,

$OCH_2\underline{CH_2}O$), 4.67 (t, J = 5 Hz, 1, OH), 5.33 (s, 2, NCH_2O), 6.66 (s, 2, NH_2), 10.81 (s, 1, NH). MS m/z 259.0470, 261.0442 (^{35}Cl,^{37}Cl). Calcd. for M^+: 259.0472, 261.0443 (^{35}Cl,^{37}Cl). UV (0.1 N HCl) max 256 nm (ε 15,200) min 221 nm (ε 2200); (0.1 N NaOH) max 268 nm (ε 13,100) min 232 nm (ε 4400). Anal. Calcd. for $C_8H_{10}ClN_5O_3$: C, 37.01; H, 3.88; N, 26.97; Cl, 13.65. Found: C, 36.95; H, 3.97; N, 26.87; Cl, 14.06.

9-[(2-Hydroxyethoxy)methyl]-8-iodoguanine (121)

To a solution of 350 mg (1.56 mmol) of 9-[(2-hydroxyethoxy)methyl]guanine (22) in 35 mL of 50% $MeOH/H_2O$ maintained at 50°C was slowly added 12 mL of MeOH containing 2.527 g (15.6 mmol) of iodine monochloride. The solution was maintained at 50°C for 18 h at which time TLC (silica, SSE) indicated the absence of starting material. 138 mg (1 mmol) of K_2CO_3 was added and volatiles were removed in vacuo. The residue was repeatedly washed with Et_2O followed by decantation until excess ICl was removed. The residue was then recrystallised twice from $EtOH/H_2O$ to give 407 mg (75%) of (121); decomposition starting at 260°C; ^1H NMR (DMSO-d^6, 200 MHz) δ 3.48 (multiplet, 4, $OCH_2\underline{CH_2}O$), 4.66 (t, J = 5 Hz, 1, OH), 5.24 (s, 2, NCH_2O), 6.58 (s, 2, NH_2), 10.66 (s, 1, NH). MS m/z 351.9854. Calcd.

for M^+: 351.9829. UV (0.1 N HCl) max 260 nm (ε 17,300) min 230 nm (ε 5200); (H_2O) max 258 nm (ε 18,100) min 224 nm (ε 2800); (0.1 N NaOH) max 270 nm (ε 15,100) min 235 nm (ε 6100). Anal. Calcd. for $C_8H_{10}IN_5O_3$: C, 27.37; H, 2.87; N, 19.95; I, 36.14. Found: C, 27.45; H, 2.93; N, 19.74; I, 36.35.

8-Amino-9-[(2-hydroxyethoxy)methyl]guanine (122)

A 200 mg (0.66 mmol) sample of 8-bromo-9-[(2-hydroxyethoxy)methyl]guanine (119) was refluxed in 10 mL of H_2O containing 0.5 mL of 95% hydrazine for 48 h. The solution was allowed to cool and the crude product collected by filtration. Recrystallisation from H_2O gave 110 mg (67%) of (122); decomposition starting at 300°C; ^1H NMR (DMSO-d^6, 100 MHz) δ 3.50 (s, 4, OCH_2CH_2O), 4.68 (s, 1, OH), 5.24 (s, 2, NCH_2O), 6.08 (s, 2, NH_2), 6.40 (s, 2, NH_2), 10.89 (s, 1, NH). MS m/z 240.0970. Calcd. for M^+: 240.0971. UV (0.1 N HCl) max 285 and 248 nm (ε 13,600 and 21,500) min 270 and 223 nm (ε 12,000 and 15,000); (0.1 N NaOH) max 255 nm (ε 20,100) min 240 nm (ε 17,000) with shoulder at 277 nm (ε 16,100). Anal. Calcd. for $C_8H_{12}N_6O_3 \cdot \frac{1}{2} H_2O$: C, 38.55; H, 5.25; N, 33.72. Found: C, 38.81; H, 5.02; N, 33.37.

9-[(2-Hydroxyethoxy)methyl]-8-methylaminoguanine (123)

A solution of 200 mg (0.66 mmol) of 8-bromo-9-[(2-hydroxyethoxy)methyl]guanine (119) in 5 mL of 50% NH_2CH_3/H_2O was sealed in a Parr steel bomb and heated at 120°C for 48 h. After cooling, the bomb was vented and volatiles were removed in vacuo. The residue was dissolved in warm H_2O and adsorbed onto a carbon column, which was washed with H_2O followed by elution of the product with 30% CH_3CN/H_2O. Removal of solvent gave 119 mg (71%) of (123) which was recrystallised from H_2O; mp 216-220°C with decomposition; ^1H NMR (DMSO-d^6, 100 MHz) δ 2.81 (d, J = 5 Hz, 3, NCH_3), 3.48 (s, 4, OCH_2CH_2O), 4.69 (s, 1, OH), 5.21 (s, 2, NCH_2O), 6.15 (q, J = 5 Hz, 1, NH), 6.26 (s, 2, NH_2), 10.30 (s, 1, NH). MS m/z 254.1125. Calcd. for M^+: 254.1127. UV (0.1 N HCl) max 287 and 248 nm (ε 9500 and 16,900) min 270 and 223 nm (ε 7300 and 4300); (0.1 N NaOH) max 259 nm (ε 13,900) min 228 nm (ε 4500) with shoulder 280 nm (ε 12,000). Anal. Calcd. for $C_9H_{14}N_6O_3$: C, 42.52; H, 5.55; N, 33.05. Found: C, 42.21; H, 5.60; N, 32.75.

9-[(2-Hydroxyethoxy)methyl]-8-dimethylaminoguanine (124)

A solution of 148 mg (0.487 mmol) of 8-bromo-9-[(2-hydroxyethoxy)methyl]guanine (119) in 5 mL of 50%

$NH(CH_3)_2/H_2O$ was sealed in a Parr steel bomb and heated at 120°C for 48 h. After cooling the bomb was vented and volatiles removed in vacuo. The residue was dissolved in warm water and adsorbed onto a carbon column, which was washed with water followed by elution of the product with 30% CH_3CN/H_2O. Removal of solvent followed by recrystallisation from H_2O gave 110 mg (85%) of (124); decomposition starting at 270°C; ^1H NMR (DMSO-d^6, 100 MHz) δ 2.86 (s, 6, $N(CH_3)_2$), 3.59 (multiplet, 4, OCH_2CH_2O), 4.68 (s, 1, OH), 5.22 (s, 2, NCH_2O), 6.42 (s, 2, NH_2) 10.48 (s, 1, NH). MS m/z 268.1281. Calcd. for M^+: 268.1283. UV (0.1 N HCl) max 288 and 258 nm (ε 10,400 and 17,500) min 280 and 231 nm (ε 10,000 and 3100); (0.1 N NaOH) max 268 nm (ε 15,200) min 231 nm (ε 3100). Anal. Calcd. for $C_{10}H_{16}N_6O_3$: C, 44.77; H, 6.01; N, 31.33. Found: C, 44.66; H, 6.01; N, 31.08.

9-[(2-Hydroxyethoxy)methyl]-8-piperidylguanine (125)

A solution of 150 mg (0.49 mmol) of 8-bromo-9-[(2-hydroxyethoxy)methyl]guanine (119) in 5 mL of 50% piperidine/H_2O was sealed in a Parr steel bomb and heated at 160°C for 48 h. After cooling the bomb was vented and volatiles removed in vacuo. The residue was dissolved in warm water and adsorbed onto a carbon

column, which was washed with water followed by elution of the product with 30% CH_3CN/H_2O. Further purification by preparative thin layer chromatography (silica gel, SSE) was required prior to recrystallisation from H_2O giving 110 mg (72%) of (125); decomposition starting at 250°C; ^1H NMR (DMSO-d^6, 100 MHz) δ 1.60 (broad s, 6, $CH_2CH_2CH_2$), 3.14 (s, 4, $N(CH_2)_2$) 3.57 (A_2B_2 multiplet, 4, OCH_2CH_2O), 4.65 (s, 1, OH), 5.17 (s, 2, NCH_2O), 6.40 (s, 2, NH_2) 10.40 (s, 1, NH). MS m/z 308.1594. Calcd. for M^+: 308.1597. UV (0.1 N HCl) max 265 nm (ε 15,500) min 235 nm (ε 3800) with shoulder at 285 nm (ε 10,700); (0.1 N NaOH) max 272 nm (ε 13,100) min 222 nm (ε 3800). Anal. Calcd. for $C_{13}H_{20}N_6O_3 \cdot \frac{1}{2} H_2O$: C, 49.20; H, 6.67; N, 26.48. Found: C, 49.16; H, 6.46; N, 26.16.

8-Hydroxy-9-[(2-hydroxyethoxy)methyl]guanine (126)

A 100 mg (0.33 mmol) sample of (119) and 270 mg (3.3 mmol) of sodium acetate were refluxed in glacial acetic acid (10 mL) for 3 h. Volatiles were removed *in vacuo*, the residue dissolved in H_2O (50 mL) and neutralised with 0.1 N NaOH. Purification was effected on a carbon column (Au-4, 15 g, elutant 20% CH_3CN/H_2O) followed by recrystallisation from $EtOH/H_2O$ to give 54 mg (65%) of (126); decomposition starting at 260°C; ^1H

NMR (DMSO-d^6, 100 MHz), δ 3.52 (multiplet, 4, OCH_2CH_2O), 4.61 (t, J = 5 Hz, 1, OH), 5.03 (s, 2, NCH_2O), 6.51 (s, 2, NH_2). 10.67 (broad s, 2, NH and NH). MS m/z 241.0812. Calcd. for M^+: 241.0811. UV (0.1 N HCl) max 247 and 293 nm (ε 11,300 and 9700) min 221 and 269 nm (ε 3500 and 4200); (0.1 N NaOH) max 256 and 281 nm (ε 10,800 and 10,200) min 230 and 273 nm (ε 4500 and 9900). Anal. Calcd. for $C_8H_{11}N_5O_4 \cdot \frac{1}{2} H_2O$: C, 38.40; H, 4.83; N, 28.00. Found: C, 38.48; H, 4.52; N, 27.94.

8-Tertiarybutyl-9-[(2-hydroxyethoxy)methyl]guanine (129)

225 mg (1 mmol) of 9-[(2-hydroxyethoxy)methyl]-guanine (22), 4 mL (64 mmol) of pivaldehyde and 5 mL of 3 M H_2SO_4 were dissolved in 35 mL of 50% acetic acid/H_2O. After careful deoxygenation separate solutions of 0.6 M $Fe(NH_4)_2(SO_4)_2.6\ H_2O$ (13 mL) and 0.37 M $K_2S_2O_8$ (21 mL) were simultaneously added over 1 h. Neutralisation with 1 N NaOH, removal of the brown precipitate by centrifugation/decantation was followed by adsorption of products onto a carbon column. Elution of the desired product (30% CH_3CN/H_2O) followed by preparative thin layer chromatography (silica, 20% $EtOH/CHCl_3$) and crystallisation from $EtOH/H_2O$ gave 118

mg (42%) of (129); decomposition starting at 200°C; ^1H NMR (DMSO-d^6, 100 MHz) δ 1.38 (s, 9, $-C(CH_3)_3$), 3.48 (s, OCH_2CH_2O), 4.62 (s, 1, OH), 5.47 (s, 2, NCH_2O), 6.46 (s, 2, NH_2) 10.60 (s, 1, NH). MS m/z 281.1488. Calcd. for M^+: 281.1488. UV (0.1 N HCl) max 259 nm (ε 15,300) min 227 nm (ε 1400) with shoulder at 277 nm (ε 11,000); (0.1 N NaOH) max 265 nm (ε 14,300) min 231 nm (ε 7700). Anal. Calcd. for $C_{12}H_{19}N_5O_3$: C, 51.24; H, 6.81; N, 24.90. Found: C, 51.10; H, 6.78; N, 24.67.

9-[(2-Hydroxyethoxy)methyl]-8-methylguanine (130)

To a deoxygenated solution of 200 mg (0.89 mmol) of 9-[(2-hydroxyethoxy)methyl]guanine (22) and 800 mg (2.9 mmol) $FeSO_4.7\ H_2O$ in 50 mL of 1 M H_2SO_4 was added a suspension of 1 g (11.1 mmol) of t-butyl hydroperoxide in 5 mL H_2O slowly over 30 m. After a further 30 m the pH of the solution was adjusted with 1 M NaOH until neutral, and the fine brown precipitate was removed by centrifugation/decantation. The solution was passed through a carbon column, which was then washed (H_2O) and the products eluted with 30% CH_3CN/H_2O. TLC (silica, $NH_4OH/iPrOH/H_2O$) indicated a 1:1 ratio of starting material (22) and desired product (130). The solvent was removed in vacuo and the residue was

resubmitted to the above conditions. TLC now showed almost total conversion to (130). After a second carbon column and recrystallisation from H_2O 135 mg (64%) of (130) were obtained; mp 304°-305°C; ^1H NMR (DMSO-d^6, 100 MHz) δ 2.40 (s, 3, CH_3), 3.46 (s, 4, OCH_2CH_2O), 4.67 (s, 1, OH), 5.36 (s, 2, NCH_2O), 6.47 (s, 2, NH_2) 10.55 (s, 1, NH). MS m/z 239.1014. Calcd. for M^+: 239.1019. UV (0.1 N HCl) max 255 nm (ε 14,200) min 225 nm (ε 3900) with shoulder 275 nm (ε 10,100); (0.1 N NaOH) max 260 nm (ε 13,400) min 230 nm (ε 6200). Anal. Calcd. for $C_9H_{13}N_5O_3$: C, 45.19; H, 5.48; N, 29.27. Found: C, 45.19; H, 5.56; N, 29.27.

9-[(2-Hydroxyethoxy)methyl]-7-methylguanine (131)

A solution of 141 mg (0.63 mmol) of 9-[(2-hydroxyethoxy)methyl]guanine (22) and 150 mg (1.25 mmol) of dimethylsulphate in 2 mL DMF was maintained with stirring at 28°C for 6 h. Ammonium hydroxide was carefully added until pH 8 was achieved and the solution diluted with acetone (10 mL) and allowed to stand overnight. The precipitate was filtered and washed with cold ethanol (2 x 2 mL). This crude product could only be recrystallised in pure form as the hydrochloride salt (from acetone/H_2O) giving 97 mg (56%); decomposition starting at 180°C; ^1H NMR (DMSO-d^6, 100

MHz) δ 3.41 (s, 3, CH_3), 3.60 (A_2B_2 multiplet, 4, OCH_2CH_2O), 5.57 (s, 2, NCH_2O), 7.29 (s, 2, NH_2). 9.26 (s, 1, H8). MS m/z (glycerol/FAB) 240 ($MH^{\oplus}$). UV (0.1 N HCl) max 256 nm (ε 17,300) min 229 nm (ε 9600) with shoulder at 276 nm (ε 12,900); (0.1 N NaOH) max 265 nm (ε 16,400) min 247 nm (ε 11,600). Anal. Calcd. for $C_9H_{13}N_5O_3$.HCl: C, 39.21; H, 5.12; N, 25.40. Found: C, 38.93; H, 5.14; N, 25.19.

REFERENCES

1. G.W. Beadle, S. Spregelman, A. Rich, R.H.C. Crick, M.W. Nirenberg, C. Yanofsky, B.F.C. Clark and K.A. Marcker in "The Molecular Basis of Life - Readings from Scientific American", R.H. Haynes and P.C. Hanawalt, Eds., Freeman, San Francisco, 1968, Chapter III.
2. C. Heidelberger in "Cancer Medicine", J.F. Holland and E. Frei, Eds., Lea and Febiger, Philadelphia, 1973.
3. a) P. Ehrlich, Chem. Ber., 42, 17 (1909).
   b) P. Ehrlich, Lancet, 2, 445 (1913).
4. a) D.D. Woods, Brit. J. Exp. Path., 21, 74 (1940).
   b) G. Domagk, Dtsch. Med. Wshr., 61, 250 (1935).
5. S. Kit, Mol. Cell. Biochem., 11, 161 (1976).
6. R.J. Whitley, L.T. Ch'ien, R. Dolin, G.J. Galasso and C.A. Alford Jr., New Engl. J. Med., 294, 1193 (1976).
7. A.T. Jamieson, J. Hay and J.H. Subak-Sharpe, J. Virology, 17, 1056 (1976).
8. T.S. Chan, Proc. Natl. Acad. Sci. U.S.A., 74, 1734 (1977).
9. H.M. Keir in "The Molecular Biology of Viruses", Cambridge University Press, Cambridge, 1968, p. 67.

10. E. de Clercq and P.F. Torrence, J. Carbohydr. Nucleosides, Nucleotides, 187 (1978).

11. W.H. Prusoff and B. Goz, in "Antineoplastic and Immunosuppressive Agents", A.C. Sartorelli and D.G. Johns, Eds., Springer-Verlag, New York, Heidelberg and Berlin, 1975, p. 272.

12. W.H. Prusoff and D.C. Ward, Biochem. Pharmacol., 25, 1233 (1976).

13. H.E. Skipper, J.A. Montgomery, J.R. Thomson and F.M. Schabel Jr., Cancer Res., 19, 425 (1959).

14. C. Temple, C.L. Kussner and J.A. Montgomery, J. Med. Pharm. Chem., 5, 866 (1962).

15. J.G. Corey and C.B. George, Biochem. Biophys. Res. Commun., 52, 496 (1973).

16. J.G. Corey, M.M. Mansell, C.B. George and D.S. Wilkinson, Arch. Biochem. Biophys., 160, 495 (1974).

17. a) M.L. Wolfrom, A.B. Foster, P. McWain, W. von Bebenburg and A. Thompson, J. Org. Chem., 26, 3095 (1961).

b) M.L. Wolfrom, P. McWain and A. Thompson, J. Org. Chem., 27, 3549 (1962).

c) M.L. Wolfrom, H.G. Gary and D. Horton, J. Org. Chem., 30, 1096 (1965).

d) M.L. Wolfrom, W. von Bebenburg, R. Pagnucco and P. McWain, J. Org. Chem., 30, 2732 (1965).

e) M.L. Wolfrom and P.J. Conigliaro, Carbohydr. Res., 20, 369 (1971).

f) M.L. Wolfrom, H.B. Bhat, P. McWain and D. Horton, Carbohydr. Res., 23, 289 (1971).

g) M.L. Wolfrom, P. McWain, H.B. Bhat and D. Horton, Carbohydr. Res., 23, 296 (1971).

h) D. Horton and S.S. Kokrady, Carbohydr. Res., 24, 333 (1972).

i) D.C. Baker, A. Cucruix, D. Horton and C. Pascard-Billy, J. Chem. Soc., Chem. Commun., 729 (1974).

18. N. Ueda, T. Kawabata and K. Takemoto, J. Heterocyclic Chem., 8, 827 (1971).

19. U.K. Pandit, W.F.A. Grose and T.A. Eggelte, Synth. Commun., 2, 345 (1972).

20. S.N. Mikhailov and J. Smrt, Collect. Czech. Chem. Commun., 40, 2353 (1975).

21. S.N. Mikhailov, S. Hynie and V.L. Florent'ev, Collect. Czech. Chem. Commun., 40, 3399 (1975).

22. V. Skarić, D. Erben, Z. Raza and D. Skarić, Croatica Chemica Acta, 52, 281 (1979).

23. V. Skarić, Z. Raza and D. Skarić, J. Chem. Soc. Perkin Trans. I, 223 (1982).

24. E. de Clercq, J. Descamps, P. de Somer and A. Holy, Science, 200, 563 (1978).

25. E. de Clercq and A. Holy, J. Med. Chem., 22, 510 (1979).

26. H.J. Schaeffer, S. Gurwara, R. Vince and S. Bittner, J. Med. Chem., 14, 367 (1971).

27. H.J. Schaeffer and D. Vogel, J. Med. Chem., 8, 507 (1965).

28. H.J. Schaeffer and C.F. Schwender, J. Pharm. Sci., 57, 1070 (1968).

29. H.J. Schaeffer and R. Vince, J. Med. Chem., 10, 689 (1967).

30. H.J. Schaeffer, R.N. Johnson, E. Odin and C. Hansch, J. Med. Chem. 13, 452 (1970).

31. E.J. Reist, A. Benitez, L. Goodman, B.R. Baker and W.W. Lee, J. Org. Chem., 27, 3274 (1962).

32. B.R. Baker and K. Hewson, J. Org. Chem., 22, 966 (1957).

33. E.J. Reist, D.F. Calkins and L. Goodman, J. Org. Chem., 32, 169 (1967).

34. M.J. Robins, J.R. McCarthy Jr. and R.K. Robins, Biochemistry, 5, 224 (1966).

35. D.H. Murray and J. Prokop, J. Pharm. Sci., 54, 1468 (1965).

36. J.R. McCarthy Jr., M.J. Robins, L. Townsend and R.K. Robins, J. Am. Chem. Soc., 88, 1549 (1966).

37. B.R. Baker, R.E. Schaub and H.M. Kissman, J. Am. Chem. Soc., 77, 5911 (1955).

38. A.D. Broom and R.K. Robins, J. Am. Chem. Soc., 87, 1145 (1965).

39. R.H. Shah, H.J. Schaeffer and D.H. Murry, J. Pharm. Sci., 54, 15 (1965).

40. J.L. York and G.A. LePage, Can. J. Biochem., 44, 331 (1966).

41. A. Bloch, M.J. Robins and J.R. McCarthy Jr., J. Med. Chem., 10, 908 (1967).

42. G.B. Elion, P.A. Furman, J.A. Fyfe, P. de Miranda, L. Beauchamp and H.J. Schaeffer, Proc. Natl. Acad. Sci. U.S.A., 74, 5716 (1977).

43. H.J. Schaeffer, L. Beauchamp, P. de Miranda, G.B. Elion, D.J. Bauer and P. Collins, Nature, 272, 583 (1978).

44. P. Collins and D.J. Bauer, J. Antimicrob. Chemother., 5, 431 (1979).

45. E. de Clercq, J. Descamps, G. Verhelst, R.T. Walker, A.S. Jones, P.F. Torrence and D. Shugar, J. Infect. Dis., 141, 563 (1980).

46. C.S. Crumpacker, L.E. Schnipper, J.A. Zaia and M.J. Levin, Antimicrob. Agts. Chemother., 15, 642 (1979).

47. K.K. Biron and G.B. Elion, Antimicrob. Agts. Chemother., 18, 443 (1980).

48. J. Harmenberg, International Conference on Human Herpes viruses, Emory University, Atlanta, Georgia, 17th-21st March, 1980 (Abstract).

49. B.M. Colby, J.E. Shaw, G.B. Elion and J.S. Pagano, J. Virology, 34, 560 (1980).

50. American Journal of Medicine. Proceedings of a Symposium on Acyclovir, July, 1982. Sponsored by Burroughs Wellcome Co. and the National Institute of Allergy and Infectious Diseases.

a. B.M. Colby, J.E. Shaw, A.K. Datta and J.S. Pagano, Reference 50, P. 77.

51. E.A. Boulter, B. Thornton, D.J. Bauer and A. Bye, Brit. Med. J., 280, 681 (1980).

52. A.S. Tyms, E.M. Scamans and H.M. Naim, J. Antimicrob. Chemother., 8, 65 (1981).

53. G.B. Elion, Reference 50, P. 7.

54. J.A. Fyfe, P.M. Keller, P.A. Furman, R.L. Miller and G.B. Elion, J. Biol. Chem., 253, 8721 (1978).

55. W.H. Miller and R.L. Miller, J. Biol. Chem., 255, 7204 (1980).

56. P.M. Keller, J.A. Fyfe, L. Beauchamp, C.M. Lubbers, P.A. Furman, H.J. Schaeffer and G.B. Elion, Biochem. Pharmacol., 30, 3071 (1981).

57. P.A. Furman, M.H. St. Clair, J.A. Fyfe, J.L. Rideout, P.M. Keller and G.B. Elion, J. Virology, 32, 72 (1979).

58. M.H. St. Clair, P.A. Furman, G.M. Lubbers and G.B. Elion, Antimicrob. Agts. Chemother., 18, 741 (1980).

59. A. Larson and B. Oberg, Antimicrob. Agts. Chemother., 19, 927 (1981).

60. P.V. McQuirt and P.A. Furman, Reference 50, P. 67.

61. P.A. Furman, P.V. McQuirt, P.M. Keller, J.A. Fyfe and G.B. Elion, Virology, 102, 420 (1980).

62. G.B. Elion, Adv. Enz. Regul., 18, 53 (1980).

63. D. Derse, Y.C. Chang, P.A. Furman and G.B. Elion, J. Biol. Chem., 256, 11447 (1981).

64. H.S. Allaudeen, J. Descamps and R.K. Sehgal, Antiviral Res., 2, 123 (1982).

65. R.L. Davidson, E.R. Kaufmann, C.S. Crumpacker and L.E. Schnippe, Virology, 113, 9 (1981).

66. Y. Nishiyama and F. Rapp, J. Gen. Virology, 45, 227 (1979).

67. H.J. Field, G. Darby and P. Wildy, J. Gen. Virology, 49, 115 (1980).

68. G. Darby, B.A. Larder, K.F. Bastow and H.J. Field, J. Gen. Virology, 48, 451 (1980).

69. J.A. Fyfe and K.K. Biron, Curr. Chemother. Infect. Dis. 2, 1377 (1980).

70. B.M. Colby, P.A. Furman, J.E. Shaw, G.B. Elion and J.S. Pagano, J. Virology, 38, 606 (1981).

71. A.K. Datta, B.M. Colby, J.E. Shaw and J.S. Pagano, Proc. Natl. Acad. Sci. U.S.A. 77, 5163 (1980).

72. J.S. Pagano and A.K. Datta, Reference 50, P. 18.

73. W.H. Burns, J.R. Wingard, W.J. Bender and R. Saral, J. Virology, 39, 889 (1981).

74. W.H. Burns, J.R. Wingard, G.R. Sandford, W.J. Bender and R. Saral, Reference 50, P. 118.

75. P. de Miranda, H.C. Krasny, D.A. Page and G.B. Elion, Reference 50, P. 31.

76. P. de Miranda, H.C. Krasny, D.A. Page and G.B. Elion, J. Pharmacol. Exp. Ther., 219, 309 (1981).

77. H.C. Krasny, P. de Miranda, M.R. Blum and G.B. Elion, J. Pharmacol. Exp. Ther., 216, 281 (1981).

78. P. de Miranda, R.J. Whitley, M.R. Blum, H.C. Krasny and G.B. Elion, Clin. Pharmacol. Ther., 26, 718 (1979).

79. P. de Miranda, S.S. Good, O.L. Laskin, H.C. Krasny, J.D. Conner and P.S. Lietman, Clin. Pharmacol. Ther., 30, 662 (1981).

80. S.S. Good and P. de Miranda, Reference 50, P. 91.

81. P. Collins and N.M. Oliver, Reference 50, P. 96.

82. E.R. Kern, J.T. Richards, L.A. Glasgow, J.C. Overall and P. de Miranda, Reference 50, P. 125.

83. N.H. Park, D. Pavan-Langston, S.L. Mclean and D.M. Albert, Antimicrob. Agts. Chemother., 15, 775 (1979).

84. D.J. Bauer, P. Collins, W.E. Tucker and A.W. Macklin, Brit. J. Ophthalmol., 63, 429 (1979).

85. H.E. Kaufman, E.D. Varnell, Y.M. Contifanto and D. Rheinstrom, Antimicrob. Agts. Chemother., 14, 842 (1978).

86. H. Shiota, S. Inoue and S. Yamane, Brit. J. Ophthalmol., 63, 425 (1979).

87. D. Pavan-Langston, D. Campbell and J. Lass, Am. J. Ophthalmol., 86, 619 (1978).

88. J.A. Oosterhuis, J. Versteeg, P.J. Kruit and B.H. Postma, Ophthalmic Res., 12, 38 (1980).

89. M.D. Trousdale and A.B. Nesburn, Reference 50, P. 155.

90. M.G. Falcon and B.R. Jones, Brit. J. Ophthalmol., 63, 422 (1979).

91. D.J. Bauer, Reference 50, P. 109.

92. M.D. Trousdale, E.C. Dunkel and A.B. Nesburn, Invest. Ophthalmol. Vis. Sci., 19, 1336 (1980).

93. J.M. Hill, B.S. Kwon, K.D. Burch, J. Deback, I. Whang, G.T. Jones, B. Luke, R. Harp, Y. Shimomura, D.S. Hull and L.P. Gangarosa, Reference 50, P. 300.

94. E.R. Kern, Reference 50, P. 100.

95. N.H. Park, D. Pavan-Langston and S.L. McLean, J. Infect. Dis., 140, 802 (1979).

96. N.H. Park, D. Pavan-Langston, M.E. Hettinger, S.L. McLean, D.M. Albert, T.S. Lin and W.H. Prusoff, J. Infect. Dis., 141, 575 (1980).

97. M.L. Landry, H.L. Lucia, G.D. Hsiung, A.D. Pronovost, P.R. Dann, M.J. August and D.R. Mayo, Reference 50, P. 143.

98. K.F. Soike, A.D. Felsenfield and P.J. Gerone, Antimicrob. Agts. Chemother., 20, 291 (1981).

99. K.F. Soike and P.J. Gerone, Reference 50, P. 112.

100 D.J. Lang and K.S. Chenng, Reference 50, P. 49.

101 L.A. Glasgow, J.T. Richards and E.R. Kern, Reference 50, P. 132.

102. B. Svennerholm, A. Vahlne and E. Lycke, Arch. Virology, 69, 43 (1981).

103. H.J. Field, S.E. Bell, G.B. Elion, A.A.Nash and P. Wildy, Antimicrob. Agts. Chemother., 15, 554 (1979).

104. R.J. Klein, A.E. Friedman-Klein and E. de Stefano, Antimicrob. Agts. Chemother., 15, 723 (1979).

105. H.J. Field and E. de Clercq, J. Gen. Virology, 56, 259 (1981).

106. R.J. Klein, E. de Stefano, A.E. Friedman-Klein and E. Brady, Antimicrob. Agts. Chemother., 19, 937 (1981).

107. D. Pavan-Langston, N.H. Park and M. Hettinger, Arch. Ophthalmol., 99, 1417 (1981).

108. B.M. Colby, J.E. Shaw, G.B. Elion and J.S. Pagano, J. Virology, 34, 560 (1980).

109. N.H. Park, D. Pavan-Langston and E. de Clercq, Reference 50, P. 151.

110. W.A. Blyth, D.A. Harbour and T.J. Hill, J. Gen. Virology, 48, 417 (1980).

111. N.H. Park, D. Pavan-Langston and S.L. McLean, J. Infect. Dis., 140, 802 (1979).

112. D. Pavan-Langston, N.H. Park and J.H. Lass, Arch. Ophthalmol., 97, 1508 (1979).

113. R.J. Klein, Reference 50, P. 138.

114. H.J. Field and G. Darby, Antimicrob. Agts. Chemother., 17, 209 (1980).

115. G. Darby, H.J. Field and S.A. Salisbury, Nature, 289, 81 (1981).

116. D.M. Coen and P.A. Schaffer, Proc. Natl. Acad. Sci. U.S.A., 77, 2265 (1980).

117. P.A. Furman, D.M. Coen, M.H. St. Clair and P.A. Schaffer, J. Virology, 40, 936 (1981).

118. D.M. Coen, P.A. Schaffer, P.A. Furman, P.M. Keller and M.H. St. Clair, Reference 50, P. 351.

119. L.E. Schnipper and C.S. Crumpacker, Proc. Natl. Acad. Sci. U.S.A., 77, 2270 (1980).

120. H.J. Field, A. McMillan and G. Darby, J. Infect. Dis., 143, 281 (1981).

121. K.K. Biron, J.A. Fyfe, J.E. Noblin and G.B. Elion, Reference 50, P. 383.

122. K.O. Smith, W.L. Kennell, R.H. Poirier and F.T. Lynd Antimicrob. Agts. Chemother., 17, 944 (1980).

123. C.S. Crumpacker, L.E. Schnipper, P. Chartrand and K.W. Knopf, Reference 50, P. 361.

124. C. McLaren, C.D. Sibrack and D.W. Barry, Reference 50, P. 376.

125. C.D. Sibrack, L.T. Gutman, C. Mclaren, C.M. Wilfert and D.W. Barry, Ped. Res., 15, 621 (1981).

126. L.E. Schnipper, C.S. Crumpacker, S.I. Marlowe, P. Kowalsky, B.J. Hershey and M.J. Levin, Reference 50, P. 387.

127. C.D. Sibrack, C. Mclaren and D.W. Barry, Reference 50, P. 373.

128. R.B. Tenser and M.E. Dunstan, Virology, 99, 417 (1979).

129. W.E. Tucker Jr., Reference 50, P. 27.

130. S.L. Spruance and G.G. Krueger, Clin. Res., 29, 82A (1981).

131. J.H. Lass, D. Pavan-Langston and N.H. Park, Am. J. Ophthalmol., 88, 102 (1979).

132. B.R. Jones, P.N. Fison, L.M. Cobo, D.J. Coster, G.M. Thompson and H.G. Falcon, Lancet, 1, 243 (1979).

133. K.R. Wilhelmus, D.J. Coster and B.R. Jones, Am. J. Ophthalmol., 91, 323 (1981).

134. J. McGill and P. Tormey, Reference 50, P. 286.

135. D.J. Coster, K.R. Wilhelmus, R. Michand and B.R. Jones, Brit. J. Ophthalmol., 64, 763 (1980).

136. P.R. Laibson, D. Pavan-Langston, W.R. Yeakly and J. Lass, Reference 50, P. 281.

137. H. Shiota, Reference 50, P. 307.

138. C. Lalau, J.A. Oosterhuis, J. Versteeg, G. van Rij, J.G.C. Renardel de Lavalette, A. Craandijk, W.R.M.J. Lamers and T. Mierlobenstoyn, Doc. Ophthalmol., 50, 287 (1981).

139. L.M.T. Collum, A. Benedict-Smith and I.B. Hillary, Brit. J. Ophthalmol., 64, 766 (1980).

140. L.M.T. Collum, P. Logan, I.B. Hillary and T. Ravenscroft, Reference 50, P. 290.

141. J.L. McGill, P. Tormey and C.B. Walker, Brit. J. Ophthalmol, 65, 610 (1981).

142. B. Young, A. Paterson and T. Ravenscroft, Reference 50, P. 311.

143. K.S. Morgan, A.H. Wander, H.E. Kaufman, E.D. Varnell and T. Creagh-Kirk, Chemotherapy, 26, 405 (1980).

144. R.F. Pass, R.J. Whitley, J.D. Whelchel, A.G. Diethelm, D.W. Reynolds and C.A. Alford, J. Infect. Dis., 140, 487 (1979).

145. C.A. Alford, Reference 50, P. 225.

146. C.D. Mitchell, B. Bean, S.R. Gentry, K.E. Groth, J.R. Boen and H.H. Balfour Jr., Lancet, 1, 1389 (1981).

147. J.D. Meyers, J.C. Wade, C.D. Mitchell, R. Saral, P.S. Lietman, D.T. Durak, M.J. Levin, A.C. Segreti and H.H. Balfour Jr., Reference 50, P. 229.

148. J.M. Goldman, P.M. Chipping, G. Agnarsdottir and D. Brigden, Lancet, 1, 820 (1979).

149. S.A. Spector, M. Hintz, C. Wyborny, J.D. Connor, R.E. Keeney and S. Liao, Reference 50, P. 275.

150. R. Saral, W.H. Burns, O.L. Laskin, G.W. Santos and P.S. Lietman, New Engl. J. Med., 305, 63 (1981).

151. J.C. Wack, B. Newton, C. Mclaren, N. Flournoy, R.E. Keeney and J.D. Meyers, Ann. Intern. Med., 96, 265 (1982).

152. R. Whitley, N. Barton, E. Collins, J. Whelchel and A.G. Diethelm, Reference 50, P. 236.

153. S. Chou, J.G. Gallagher and T.C. Merigan, Lancet, 1, 1392 (1981).

154. J.W.M. van der Meer, J. Thompson, W.D. Tan and J. Versteeg Lancet, 2, 473 (1980).

155. J.W.M. van der Meer and J. Versteeg, Reference 50, P. 271.

156. P.J. Selby, B. Jameson, J.G. Watson, G. Morgenstern, R.L. Powles, H.E.M. Kay, R. Thornton, H.M. Clink and T.J. McElwain, Lancet, 2, 1267 (1979).

157. N.A. Peterslund, J. Ipsen, H. Schonheyder, K. Seyer-Hansen, V. Esmann and H. Juhl, Lancet, 2, 827 (1981).

158. J.L. Sullivan, K.S. Byron, F.E. Brewster, K. Sakamoto, J.E. Shaw and J.S. Pagano, Reference 50, P. 262.

159. H.H. Balfour Jr., B. Bean, C.D. Mitchell, G.W. Sachs, J.R. Boen and C.K. Edelman, Reference 50, P. 241.

160. J.C. Wade, M. Hintz, R.W. McGuffin, S.C. Springmeyer, J.D. Connor and J.D. Meyers, Reference 50, P. 249

161. T.R. Cupps, S.E. Strans and T.A. Waldmann, Am. J. Med., 70, 882 (1981).

162. S.A. Plotkin, S.E. Starr and C.K. Bryan, Reference 50, P. 257.

163. S.L. Spruance and C.S. Crumpacker, Reference 50, P. 315.

164. L. Corey, J.K. Benedetti, C.W. Critchlow, M.R. Remington, C.A. Winter, A.L. Fahnlander, K. Smith,

D.L. Slater, R.E. Keeney, L.G. Davis, M.A. Hintz, J.D. Connor and K.K. Holmes, Am. J. Med., Reference 50, P. 326.

165. A.P. Fiddian, A.M. Halsas, B.R. Kinge, A.E. Nilsen and K. Wilkstrom, Reference 50, P. 335.

166. R.C. Reichman, M. Ginsberg, E. Barrett-Connor, C. Cyborny, J.D. Connor, D. Redfield, M.C. Savoia, D.D. Richman, M.N. Oxman, P.S. Dandliker, G.J. Badger, T. Ashikaga and R. Dolin, Reference 50, P. 338.

167. A. Mindel, M.W. Adler, S. Sutherland and A.P. Fiddian, Reference 50, P. 347.

168. A.J. Nahmias, G. Kerrick, H.L. Keyserling, G. Davis, J. Alexander, L. Corey, V. Hatcher, J. Luby, J. Mills and S. Sacks, Reference 50, P. 342.

169. S.A. Spector, M. Tyndall and E. Kelley, Reference 50, P. 36.

170. R.F. Schinazi and A.J. Nahmias, Reference 50, P. 54.

171. K.K. Biron and G.B. Elion, Reference 50, P. 294.

172. W.J. O'Brien and J.L. Taylor, Reference 50, P. 294.

173. R.B. van Dyke, J.D. Connor, C. Wyborny, M. Hintz and R.E. Keeney, Reference 50, P. 172.

174. R.J. Whitley, M.R. Blum, N. Barton and P. de Miranda, Reference 50, P. 165.

175. R.J. Whitley, M.R. Blum, N. Barton and P. de Miranda, Clin. Pharmacol. Ther., 26, 718 (1979).

176. D. Bridgen, A. Bye, A.S.E. Fowle and H. Rogers, J. Antimicrob. Chemother., 7, 399 (1981).

177. P. de Miranda, S.S. Good, H.C. Krasny, J.D. Connor, O.L. Laskin and P.S. Lietman, Clin. Pharmacol. Ther. 30, 662 (1981).

178. A.S. Yeager, Reference 50, P. 205.

179. M. Hintz, J.D. Connor, S.A. Spector, M.R. Blum, R.E. Keeney and A.S. Yeager, Reference 50, P. 210.

180. O.L. Laskin, P. de Miranda, D.H. King, D.A. Page, J.A. Longstreth, L. Rocco and P.S. Lietman, Antimicrob. Agts. Chemother., 21, 804 (1982).

181. P. de Miranda, R.J. Whitley, M.R. Blum, R.E. Keeney, N. Barton, D.M. Cocchetto, S. Good, G.P. Hemstreet III, L.E. Kirk, D.A. Page and G.B. Elion, Clin. Pharmacol. Ther., 26, 718 (1979).

182. S.A. Spector, J.D. Connor, M. Hintz, R.P. Quinn, M.R. Blum and R.E. Keeney, Antimicrob. Agts. Chemother., 19, 608 (1981).

183. P.S. Lietman, Reference 50, P. 193.

184. O.L. Laskin, J.A. Longstreth, R. Saral, P. de Miranda, R. Keeney and P.S. Lietman, Antimicrob. Agts. Chemother., 21, 393 (1982).

185. O.L. Laskin, J.A. Longstreth, A. Whelton, L. Rocco, P.S. Lietman, H.C. Krasny and R.E. Keeney, Clin. Pharmacol. Ther., 31, 594 (1982).

186. O.L. Laskin, R. Saral, W.H. Burns, C.M. Angelopulos and P.S. Lietman, Reference 50, P. 221.

187. P. de Miranda, S.S. Good, H.C. Krasny, J.D. Connor. O.L. Laskin and P.S. Lietman, Reference 50, P. 215.

188. M.R. Blum, S.H.T. Liao and P. de Miranda, Reference 50, P. 186.

189. R.E. Keeney, K.E. Kirk and D. Bridgen, Reference 50, P. 176.

190. D. Bridgen, A.E. Rosling and N.C. Woods, Reference 50, P. 182.

191. A. Kossel, Arch. Anat. U. Physiol. Physiol. Abt., 181 (1891).

192. E. Volkin, J.X. Khum and W.E. Cohn, J. Am. Chem. Soc., 73, 1533 (1951).

193. C. Tamm, M.E. Hodes and E. Chargaff, J. Biol. Chem., 195, 45 (1952).

194. A.M. Michelson in "The Chemistry of Nucleosides and Nucleotides", Academic Press, N.Y., 1963. P. 26.

195. P.A. Levene and W.A. Jacobs, J. Biol. Chem., 12, 411 (1912).

196. P.A. Levene, J. Biol. Chem., 48, 119 (1921).

197. S.J. Thannhauser and B. Ottenstein, Z. Physiol. Chem., 114, 39 (1921).

198. S.J. Thannhauser and G. Blanco, Z. Physiol. Chem., 116, 161 (1926).

199. P.A. Levene and E. Jorpes, J. Biol. Chem., 81, 575 (1929).

200. C.A. Dekker, A.M. Michelson and A.R. Todd, J. Chem. Soc., 947 (1953).

201. H. Shapiro and E. Chargaff, Biochim. Biophys. Acta, 26, 596 (1957).

202. H. Shapiro and E. Chargaff, Biochim. Biophys. Acta, 26, 608 (1957).

203. P.T. Gilham and H.G. Khorana, J. Am. Chem. Soc., 80, 6212 (1958).

204. G.W. Kenner in "The Chemistry and Biology of Purines", G.E.W. Wolstenholme and C.M. O'Connor, Eds., Little and Brown Co., Boston, 1957, P. 312.

205. H.S. Isbell and H.L. Frush, J. Org. Chem., 23, 1309 (1958).

206. B. Capon, Chem. Rev., 69, 407 (1969).

207. P. Karrer and J. ter Kuile, Helv. Chim. Acta, 5, 870 (1922).

208. C.A. Dekker, Ann. Rev. Biochem., 29, 463 (1960).

209. S.G.A. Alivisatos and L. La Mantia, Biochem. Biophys. Res. Commun. 2 164 (1960).

210. S.G.A. Alivisatos, L. La Mantia and B.L. Matijevitch, Biochim. Biophys. Acta, 58, 209 (1962).

211. E.R. Garrett and L.J. Hanka, Am. Pharm. Assn. J. Sci. Ed., 49, 526 (1960).

212. E.R. Garrett, J. Am. Chem. Soc., 82, 827 (1960).

213. H. Venner, Z. Physiol. Chem., 339, 14 (1964).

214. H. Venner, Z. Physiol. Chem., 344, 189 (1966).

215. E.R. Garrett, T. Suzuki and D.J. Weber, J. Am. Chem. Soc., 86, 4460 (1964).

216. E.R. Garrett, P.B. Chemburkar and T. Suzuki, Chem. Pharm. Bull., 13, 1113 (1965).

217. R. Shapiro and S. Kang, Biochemistry, 8, 1809 (1969).

218. R. Shapiro and M. Danzig, Biochemistry, 11, 23 (1972).

219. E.R. Garrett, J.K. Seydel and A.J. Sharpen, J. Org. Chem., 31, 2219 (1966).

220. H. Tománková and J. Zýka, Microchem. Journal, 25, 281 (1980).

221. H. Lonneberg, Tetrahedron, 38, 1517 (1982).

222. E.R. Garrett and P.J. Mehta, J. Am. Chem. Soc., 94, 8532 (1972).

223. J. Baddiley, J.G. Buchanan and G.O. Osbourne, J. Chem. Soc., 3606 (1958).

224. F. Jorden and H. Niv, Nucl. Acid Res., 4, 697 (1977).

225. H. Lonneberg and P. Lehikoinen, Nucl. Acid Res., 10, 4339 (1982).

226. P.R. Young and W.P. Jencks, J. Am. Chem. Soc., 99, 8238 (1977).

227. C.C. Jones, M.L. Sinnott and I.J.L. Souchard, J. Chem. Soc. Perkin II, 1191 (1977).

228. J.A. Zoltewicz, D.F. Clark, T.W. Sharpless and G. Grahe, J. Am. Chem. Soc., 92, 1741 (1970).

229. J.A. Zoltewicz and D.F. Clark, J. Org. Chem., 37, 1193 (1972).

230. L. Hevesi, E. Wolfson-Davidson, J.B. Nagy, O.B. Nagy and A. Bruylants, J. Am. Chem. Soc., 94, 4715 (1972).

231. R.P. Panzica, R.J. Rousseau, R.K. Robins and L.B. Townsend, J. Am. Chem. Soc., 94, 4708 (1972).

232. B. Mety and P. Claverie, Biochim. Biophys. Acta, 353, 28 (1974).

233. R. Romero, R. Sstein, H.G. Bull and E.H. Cordes, J. Am. Chem. Soc., 100, 7620 (1978).

234. H. Lonneberg and A. Koskinen, Acta. Chem. Scand., A34, 181 (1980).

235. H. Lonneberg, Acta. Chem. Scand., A35, 317 (1981).

236. H. Lonneberg, Acta. Chem. Scand., A34, 703 (1980).

237. H. Lonneberg, R. Kappi and E. Heikkinen, Acta. Chem. Scand., B35, 589 (1981).

238. R.M. Izatt, J.J. Christensen and J.H. Rytting, Chem. Rev., 71, 439 (1971).

239. G.L. Eichhorn in "Inorganic Biochemistry", G.L. Eichhorn Ed., Elsevier, Amsterdam, 1973, P. 1191.

240. R.B. Martin and G.H. Mariam, "Metal ions in Biological Systems", H. Sigel Ed., Dekker, N.Y. 1979, Vol. 8, P. 57.

241. A. Kossel and A. Neumann, Ber. Dtsch. Ges., 26, 2753 (1893).

242. H. Bredereck and G. Müller, Ber. Dtsch. Ges., 72, 115 (1939).

243. V.W. Pollmann and G. Schramm, Z. Naturforsch., 16B, 673 (1961).

244. P.O.P. Ts'O in "Basic Principles in Nucleic Acid Chemistry", Vol. I, P.O.P. Ts'O, Ed. Academic Press, N.Y., 1974. P. 512.

245. Y. Furukawa, K. Kobayashi, Y. Kanai and M. Monjo, Chem. Pharm. Bull., 13, 1273 (1965).

246. D.M.G. Martin, C.B. Reese and G.F. Stephenson, Biochemistry, 7, 1406 (1968).

247. J.B. Gin and C.A. Dekker, Biochemistry, 7, 1413 (1968).

248. J.L. York, J. Org. Chem., 46, 2171 (1981).

249. G. Etzold, R. Hintsche, G. Kowollik and P. Langen, Tetrahedron, 27, 2463 (1971).

250. A.M. Michelson, Tetrahedron, 2, 333 (1948).

251. D.M. Brown, G.D. Fasman, D.I. Magrath and A.R. Todd, J. Chem. Soc., 1448 (1954).

252. M.J. Robins and R.K. Robins, J. Am. Chem. Soc., 86, 3585 (1964).

253. I. Wempen, I.L. Doerr, L. Kaplan and J.J. Fox, J. Am. Chem. Soc., 82, 1624 (1960).

254. M. Ikehara and H. Tada, Chem. Pharm. Bull., 14, 197 (1966).

255. M.J. Robins and G.L. Basom, Can. J. Chem., 51, 3161 (1973).

256. P. Sporns, Ph.D. Dissertation, The University of Alberta, (1977).

257. G.G. Cross, M.Sc. Thesis, The University of Alberta, (1979).

258. a) H.S. Isbell and H.L. Frush, J. Res. Natl. Bur. Standards, 24, 125 (1940).

b) E. Dyer, P.J. Glaudermans, M.J. Koch and R.H. Marchessault, J. Chem. Soc., 3361 (1962).

c) C.A. Bunton, T.A. Lewis, D.R. Llewellyn and C.A. Vernon, J. Chem. Soc., 4419 (1955).

d) C. Armour, C.A. Bunton, S. Patai, L.H. Selman and C.A. Vernon, J. Chem. Soc., 412 (1961).

e) B.E.C. Banks, Y. Meinwald, A.J. Rhind-Tutt, I. Shaft and C.A. Vernon, J. Chem. Soc., 3240 (1961).

f) W.G. Overend, C.W. Rees and J.S. Sequerra, J. Chem. Soc., 3429 (1962).

g) C. Bamford, B. Capon and W.G. overend, J. Chem. Soc., 5138 (1962).

h) R.J. Withey and E. Whalley, Can. J. Chem., 41, 1849 (1963).

i) T.E. Timell, Chem. Ind., 503 (1964).

j) T.E. Timell, W. Euterman, F. Spencer and E.J. Soltes, Can. J. Chem., 43, 2296 (1965).

k) M.D. Saunders, Diss. Abstr. B., 28, 4507 (1968).

l) M.S. Feather and J.F. Harris, J. Org. Chem., 30, 153 (1965).

m) B. Capon and D. Thacker, J. Chem. Soc., 185 (1967).

n) J.N. Bemiller and E.R. Doyle, Carbohydr. Res., 20, 23 (1971).

259. C. Altona and M. Sundaralingham, J. Am. Chem. Soc., 94, 8205 (1972).

260. M.J. Robins and M. MacCoss, unpublished results

261. M. Smith, G.I. Drummond and H.G. Khorana, J. Am. Chem. Soc., 83, 698 (1961).

262. G.M. Tener, H.G. Khorana, R. Markham and E.H. Pol, J. Am. Chem. Soc., 80, 6223 (1958).

263. K. Isono, P.F. Crain and J.A. McCloskey, J. Am. Chem. Soc., 97, 943 (1975).

264. J. Cadet and R. Theoule, J. Am. Chem. Soc., 96, 6517 (1974).

265. H. Venner, Hoppes Eylers' Z. fur Phys. Chem., 344, 189 (1966).

266. A. Albert in "Physical Methods in Heterocyclic Chemistry", A.R. Katritzky Ed., Academic Press, N.Y., Vol. 1, 1963, P. 2.

267. H. Lonneberg and R. Kappi, Tetrahedron, 36, 913 (1980).

268. M.L. Sinnott and S.G. Withers, Biochem. J., 143, 751 (1974).

269. R. Stewart and M.G. Harris, J. Org. Chem., 43, 3123 (1978).

270. E.D. Becker, H.T. Miles and R.B. Bradley, J. Am. Chem. Soc., 87, 5575 (1965).

271. a) W. Pfleiderer, Ann. 647, 167 (1961).
b) M. Tsuboi, Y. Kyogoku and T. Schimawanchi, Biochim. Biophys. Acta, 55, 1 (1962).

272. J.A. Montgomery and H.J. Thomas, J. Am. Chem. Soc., 87, 5442 (1965).

273. J.A. McCloskey, J.H. Futrell, T.A. Elwood, K.H. Scram, R.P. Panzica and L.B. Townsend, J. Am. Chem. Soc., 95, 5762 (1973).

274. N.J. Leonard and R.A. Laursen, Biochemistry, 4, 354 (1965).

275. T. Itaya and H. Matsumoto, Tetrahedron Lett., 4047 (1978).

276. T. Itaya and K. Ogawa, Tetrahedron Lett., 2907 (1978).

277. S. Nakatsuka, T. Ohgi and T. Goto, Tetrahedron Lett. 2579 (1978).

278. T. Saito and T. Fujii, J. Chem. Soc. Chem. Commun., 135 (1979).

279. T. Itaya, T. Wantanabe and H. Matsumoto, J. Chem. Soc. Chem. Commun., 1158 (1980).

280. T. Itaya and T. Harada, Heterocycles, 19, 687 (1982).

281. R.E. Notari, D.T. Witiak, J.L. de Young and A. Jeng, J. Med. Chem., 15, 1207 (1972).

282. T. Fujii and T. Saito, Heterocyles, 17, 117 (1982).

283. H.G. Bull, J.P. Ferraz, E.H. Cordes, A. Ribbi and R. Apitz-Castro, J. Biol. Chem., 253, 5186 (1978).

284. E.H. Cordes and H.G. Bull in "Transition States of Biochemical Processes", R.D. Gandour and R.L. Schowen Eds., Plenum Press, N.Y., 1978, P. 429.

285. V.M. Clark, A.R. Todd and J. Zussman, J. Chem. Soc., 2952 (1951).

286. E.M. Arnett, Prog. Phys. Org. Chem., 1, 223 (1963).

287. K.K. Ogilvie and M.F. Gillen, Tetrahedron Lett., 327 (1980).

288. A.C. Schroeder, R.G. Hughes Jr. and A. Bloch, J. Med. Chem., 24, 1078 (1981).

289. a) U. Niedballa and H. Vorbrüggen, J. Org. Chem., 39, 3654 (1974).

b) U. Niedballa and H. Vorbrüggen, J. Org. Chem., 39, 3660 (1974).

c) U. Niedballa and H. Vorbrüggen, J. Org. Chem., 39, 3664 (1974).

d) U. Niedballa and H. Vorbrüggen, J. Org. Chem., 39, 3668 (1974).

e) U. Niedballa and H. Vorbrüggen, J. Org. Chem., 39, 3672 (1974).

290. H. Vorbrüggen, K. Krolikiewicz and B. Bennua, Chem. Ber., 114, 1234 (1981).

291. A. Rosowsky and S.H. Kim, Abstracts of papers, 181st National Meeting of the American Chemical Society, Atlanta, Mar.-Apr., 1981, MEDI 34.

292. A. Rosowsky, S.H. Kim and M. Wick, J. Med. Chem., 24, 1177 (1981).

293. J.E. McCormick and R.S. McElhinney, J. Chem. Res. Synop., 12 (1981).

294. M. Senkus, J. Am. Chem. Soc., 68, 734 (1946).

295. G.E. Keyser, J.D. Bryant and J.R. Barrio, Tetrahedron Lett., 35, 3263 (1979).

296. J.D. Bryant, G.E. Keyser and J.R. Barrio, J. Org. Chem., 44, 3733 (1979).

297. J.R., Barrio, J.D. Bryant and G.E. Keyser, J. Med. Chem., 23, 572 (1980).

298. T. Nishimura, B. Shimizu and I. Iwai, Chem. Pharm. Bull., 11, 1470 (1963).

299. T. Nishimura, B. Shimizu and I. Iwai, Chem. Pharm. Bull., 12, 1471 (1964).

300. E. Wittenburg, Chem. Ber., 101, 1095 (1968).

301. M.J. Robins in "Nucleoside Analogues", Eds. R.T. Walker, E. de Clercq and F. Eckstein, Plenum Press, N.Y., 1979, P. 193.

302. E.K. Ryu and M. MacCoss, J. Org. Chem., 46, 2820 (1981).

303. A.R. Katritzky and A.J. Waring, Chem. Ind. (London), 695 (1962).

304. D.J. Brown and J.M. Lyall, Aust. J. Chem., 15, 851 (1962).

305. P. Brookes and P.D. Lawley, J. Chem. Soc., 1348 (1962).

306. T. Ueda and J.J. Fox, J. Am. Chem. Soc., 85, 4024 (1963).

307. L.B. Townsend in "Nucleoside Analogues", Eds. R.T. Walker, E. de Clercq and F. Eckstein, Plenum Press, N.Y., 1979, P. 193.

308. W.W. Lee, A.P. Martinez, L. Goodman and D.W. Henry, J. Org. Chem., 37, 2923 (1972).

309. R.B. Simpson, J. Am. Chem. Soc., 86, 2059 (1964).

310. S. Mansy and R.S. Tobias, J. Am. Chem. Soc., 96, 6874 (1974).

311. T.P. Johnston, L.B. Holum and J.A. Montgomery, J. Am. Chem. Soc., 80, 6265 (1958).

312. J.W. Jones and R.K. Robins, J. Am. Chem. Soc., 85, 193 (1963).

313. T. Itaya, H. Matsumoto and T. Watanabe, Chem. Pharm. Bull, 30, 86 (1982).

314. A. Parkin and M.R. Harnden, J. Heterocyclic Chem., 19, 33 (1982).

315. R.E. Holmes and R.K. Robins, J. Am. Chem. Soc., 86, 1242 (1964).

316. M. Ikehara, H. Tada and K. Muneyama, Chem. Pharm. Bull., 13, 1140 (1965).

317. R.A. Long, R.K. Robins and L.B. Townsend, J. Org. Chem., 32, 2751 (1967).

318. M. Ikehara, S. Uesugi and M. Kaneko, J. Chem. Soc. Chem. Commun., 1, 17 (1967).

319. D. Lipken, F.B. Howard, D. Notwotny and M. Sano, J. Biol. Chem., 238, PC 2249 (1963).

320. R.E. Holmes and R.K. Robins, J. Am. Chem. Soc., 87, 1772 (1965).

321. H. Steinmaus, I. Rosenthal and D. Elad, J. Org. Chem., 36, 3594 (1971).

322. M. Maeda, K. Nushi and Y. Kawazoe, Tetrahedron, 30, 2677 (1974).

323. M. Ikehara, W. Limn and T. Fukui, Chem. Pharm. Bull., 25, 2702 (1977).

324. R. Pless, L. Dudycz, R. Stolarsky and D. Shugar, Z. Naturforsch., 33c, 902 (1978).

325. J.L. Kelley, J.E. Kelsey, W.R. Hall, M.P. Krochmal and H.J. Schaeffer, J. Med. Chem., 24, 753 (1981).

326. J.L. Kelley, M.P. Krochmal and H.J. Schaeffer, J. Med. Chem., 24, 472 (1981).

327. M.J. Robins and P.W. Hatfield, Can. J. Chem., 60, 547 (1982).

328. E. de Clercq, J. Balzarini, P.F. Torrence, M.P. Mertes, C.L. Schmidt, D. Shugar, P.J. Barr, A.S. Jones, G. Verhelst and R.T. Walker, Molec. Pharmacol., 19, 321 (1981).

329. J.G. Niedzwicki, S.H. Chu, M.H. el Kouni and S. Cha, The Pharmacologist, 22, 241 (1980).

330. J.G. Niedzwicki, M.H. el Kouni, S.H. Chu and S. Cha, Biochem. Pharmacol., 30, 2097 (1981).

331. J.G. Niedzwicki, S.H. Chu, M.H. el Kouni and S. Cha, Biochem. Pharmacol., 31, 1857 (1982).

332. G.D. Birnie, H. Kroeger and C. Heidelberger, Biochemistry, 2, 566 (1963).

333. C. Heidelberger, G.D. Birnie, J. Boohar and D. Wentland, Biochim. Biophys. Acta, 76, 315 (1963).

334. J.J. Windheuser and J. Jato, J. Pharm. Sci., 61, 1669 (1972).

335. Estimated from that of guanosine (pka 1.6).[266]

336. J.A. Haines, C.B. Reese and Lord Todd, J. Chem. Soc., 5281 (1962).

337. L.B. Townsend and R.K. Robins, J. Am. Chem. Soc., 85, 242 (1963).

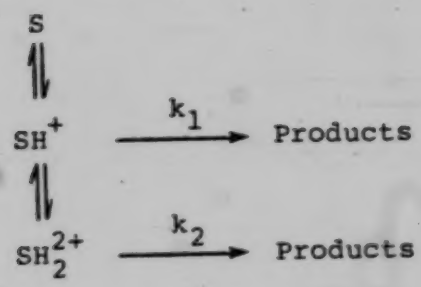
338. Crystalline, stable Schiff bases are readily prepared e.g. "Eschenmosers Salt" J. Schreiber, H. Maag, N. Hashimoto and A. Eschenmoser, Angew. Chem. Int. Ed. Engl., 10, 330 (1971).

339. A.I. Vogel "Practical Organic Chemistry" 3rd Edition, Longman, London, 1970.

340. D.D. Perrin, W.L.F. Armarego and D.R. Perrin, "Purification of Laboratory Chemicals" 2nd Edition, Pergamon Press, Toronto, 1980.

APPENDIX

Scheme II (for clarity charges have been omitted eg. a proton is represented by [H]).



$$K_{a_2} = \frac{[S][H]}{[SH]}$$

$$K_{a_1} = \frac{[SH][H]}{[SH_2]}$$

$$\text{Rate} = k_{obs}[S_{TOT}] = k_1[SH] + k_2[SH_2]$$

$$k_{obs}([S]+[SH]+[SH_2]) = k_1[SH] + k_2[SH_2]$$

$$k_{obs}\left([S]+\frac{[H][S]}{K_{a_2}}+\frac{[H]^2[S]}{K_{a_1}K_{a_2}}\right) = \frac{k_1[S][H]}{K_{a_2}} + \frac{k_2[H]^2[S]}{K_{a_1}K_{a_2}}$$

$$k_{obs}\left(1+\frac{[H]}{K_{a_2}}+\frac{[H]^2}{K_{a_1}K_{a_2}}\right) = \frac{k_1[H]}{K_{a_2}} + \frac{k_2[H]^2}{K_{a_1}K_{a_2}}$$

$$k_{obs} = \frac{K_{a_1}[H]\left(k_1 + \frac{k_2[H]}{K_{a_1}}\right)}{K_{a_1}K_{a_2} + [H]K_{a_1} + [H]^2}$$